# LYMPHATICS
# AND LYMPH CIRCULATION

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## PHYSIOLOGY AND PATHOLOGY

BY

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TO THE MEMORY

OF

ALEXANDER KORÁNYI

# PREFACE

Opinions regarding the importance of lymph circulation have undergone a remarkable evolution. Whilst, at the end of the past century, the function of the lymphatic system was generally regarded as fundamentally important, it seems that no such importance has been attached to it during the last decades although the number of publications dealing with lymph circulation has by no means decreased. Whereas, in the past, a decisive importance was ascribed to lymph circulation in matters as important and fundamental as, for instance, the mechanism of oedema formation, investigation is nowadays more concerned with matters of detail and, although a great number of new facts has been discovered by different authors, in default of a synthesis — a comprehensive uniform concept — no real progress in the investigation of lymph circulation has been made.

Although we have a very high opinion of the works published on the problems of lymph circulation in the last decades, we think that — for different reasons — they are not fully satisfactory. The monograph of Rouvière and Valette, issued in 1937, originates from an epoch when numerous problems of water and salt balance were still unsolved. This applies more or less also to the monograph of Drinker and Yoffey, published in 1941. — Fifteen years have passed since, and our knowledge has much increased owing to a number of new discoveries. Their synthesis and uniform criticism seemed to be absolutely necessary. The recently-published work of Zhdanov could not solve this task since its principal purpose was of a morphological nature.

When, 8 years ago, we began to concern ourselves with the problems of lymph circulation we returned, as a matter of fact, to the question treated so intensively by the classics of medical science (Ludwig, Heidenhain, Cohnstein, Korányi, Starling, etc.) at the end of the past century, namely to the question of oedema. One of us (Rusznyák), in a work on the origin of oedemas, pointed out as early as in 1938 that it was impossible to offer a satisfactory explanation of oedema formation without due regard to the lymphatic system.

Our researches have led to the results described in the present book

which go beyond the question of the pathogenesis of oedemas and illustrate the great importance of lymph circulation under different physiological and pathological conditions.

Our results are due not only to the fact that our knowledge concerning the movement of water and dissolved substances have been extraordinarily increased in the last decades, but in the first place to that uniform concept which is associated with the name of Alexander Korányi, i.e. the functional conception of biological phenomena.

Essentially, the notion of function means in medical science a unity of organism and environment. Korányi's great achievement consisted in the abandonment of a merely morphological way of thinking: he considered the human body and the function of its organs in their aspects of movement and change and examined the way in which the organism satisfied the requirements demanded from it. We think that the application of this principle is fruitful also for the elucidation of the problems of lymph circulation.

The problems treated by us in this work have presented themselves at the bedside. In order to solve them we made use of the so-called theoretical sciences. Also in this connection were we endeavoured to follow in Alexander Korányi's footsteps who had repeatedly emphasized the well-known demand of the great French physiologist, Claude *Bernard, that "the medicine of the future should, in its scientific part,* consist in applied physiology". This unity of theoretical and practical medicine, or — as Pavlov called it — their "fruitful alliance" means also the unity of the entire medical science. The principal aim of medical activity and therapy is the prevention and treatment of diseases. We think that from this point of view it would be a mistake to separate clinical and theoretical work since, in daily practice, a great many histological, chemical and physical methods are employed. *Medical practice* is, however, not identical with the cultivation of *medical science* although the latter has the same aims as medical practice, it serves *them by the discovery of new facts and methods.*

These principles formed the basis of Alexander Korányi's activity, and we too have tried to serve these aims by examining the problems of lymph circulation.

We should not have been able to carry out our investigations without the far-reaching support of the Hungarian Academy of Sciences which assured the financial basis of our investigations and made, more-

over, the edition of this book possible. We are also indebted to all collaborators who participated in our researches and lent us valuable help, further to the scholars who placed unpublished illustrations and data at our disposal. Finally, we have to thank the Publishing House of the Hungarian Academy of Sciences for its careful work.

# PREFACE TO THE ENGLISH EDITION

Although only a comparatively short period has elapsed since the publication of the Hungarian, the German and the Russian editions of our monograph, the present work is not a simple translation of the Hungarian original. Induced by the new results obtained from our own and others' experiments we deemed a partial revision of the text necessary. Some chapters have been wholly re-written (absorption from serous cavities, composition of lymph), new chapters inserted (e.g. lymphatics of the salivary glands, function of lymph hearts, etc.) and others completed by recent data. A few illustrations have been replaced by better ones, and several voluminous tables which disturbed the continuity of the text, have been replaced by illustrations.

We hope our work will be kindly received by the reader.

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# INTRODUCTION

The lymph vascular system is one of tubes co-ordinated with and, to a certain extent, complementary to the blood vascular system. This system is found only in vertebrates and consists in the higher orders of a network of closed lymph capillaries and efferent lymphatics. In this system of tubes a fluid — the lymph — is flowing which, as Drinker and Yoffey in the preamble of their monograph put it, "is on its way from and to the blood". Therefore, even if the lymph vascular system is merely one of efferent tubes, we feel justified in speaking of lymph circulation.

The passage of lymph from the lymph capillaries through the collecting and efferent lymph trunks and its drainage into the large veins is, in our opinion, only a part of this circulation. The process begins, properly speaking, with capillary filtration where water and dissolved molecules escape from the blood capil laries and pass into the interstitia space. The second step according t [illegible]

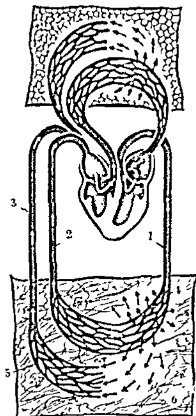



*Fig. 1.* Schematic representation of blood and lymph circulation

1 = Artery, 2 = Vein, 3 = Lymphatic; 4 = Blood capillaries, 5 = Lymphatic capillaries; 6 = Interstitial space

mixing with extracellular fluid and arrival to the wall of the lymphatic capillaries. In the following phase of this circulation the fluid leaks through the wall of the lymphatic capillaries and so passes into the closed tube system: the lymph vascular system wherefrom it returns to the blood vessels. It is in this sense that the term "lymph circulation" is understood by us. Although, in the stricter sense of the word, we call lymph only the fluid which has already gained access to the lymphatic

system, capillary filtration and diffusion in the connective tissue also contribute to the formation of this lymph. It is for this reason that we will study this process in the present book as a whole, and examine not only the questions of lymph flow but also the problems of capillary filtration and diffusion in the connective tissue. We shall see that these intricate processes can be explained only from a uniform point of view.

FIRST PART

# ORIGIN AND ARCHITECTURE OF LYMPHATIC SYSTEM

# CHAPTER I

# HISTORY OF THE DISCOVERY OF LYMPHATICS AND LYMPH CIRCULATION

Only comparatively few medico-historical works have dealt with the discovery of lymph circulation and lymphatic system. There are, however, as Bartels (1909) observes, few domains of medical science with a more interesting and fascinating history, since — strictly, speaking — there can be no question of the discovery of other systems, e.g. of the arterial, venous or nervous systems etc., as practically, they have been known from times immemorial; the most that can be said is that knowledge and views relating to them were unclear and erroneous. The lymphatic system, on the other hand, was actually "discovered" as will be shown hereinafter. Details of this discovery and the discussions connected with it were given by two works towards the end of the past century, i.e. the publications of His (1871), and Tigerstedt (1895). Worthy of note among the recent treatises of an historical nature is Zhdanov's (1919b) work on the discovery of the thoracic duct and the more important lymphatic trunks.

The discovery of lymphatic vessels, the recognition of a lymphatic system, dates back to the 17th century.

Descriptions in the medical literature of the antiquity make it probable that lymphatic vessels had been observed and were known in those times. Already Hippocrates spoke of "white blood"; also Aristotle is cited as having described structures containing a colourless fluid, and it is quite certain that the physicians of the Alexandrine school saw and described the Ductus lactei both in humans and animals. But in the Middle Ages, when the development of medical science had stopped and even declined in Europe, such knowledge fell into complete oblivion. It is no mere chance that lymphatics and lymph circulation were discovered anew after almost two thousand years, in an epoch when medicine and other natural sciences were approaching a new golden age. That it was really not a question of mere chance is evident also from the fact that almost all important discoveries were made simultaneously by two or more scholars who worked independently of one another.

The function of the lymphatic system remained essentially unknown for a long time even after the discovery of its existence. This was due to two reasons. The first was a lack of suitable methods; the contents of the smaller lymphatic vessels are colourless and their walls so thin as to be invisible to the naked eye; the second reason was a lack of interest because — given the knowlege of those times — scientists were really at a loss as to what to make of the discovery.

The glory of having discovered the lymphatic vessels belongs undoubtedly to Asellius (1627), who worked at Milan, a new centre of civilization. As reported by Bartels (1909), he performed on the 23rd of July 1622, at the request of some of his friends, a vivisection on a well-fed dog with a view to demonstrating the course of the Nervi recurrentes and the movements of the diaphragm. He was opening the abdominal cavity and pulling the stomach and intestines aside when, suddenly, he was astonished to behold ramifying vessels in the mesentery which were filled with a white fluid. Taking them for nerves, he paid no attention to them at first, but later the nerves came into sight and could be clearly distinguished from these bundles. "Surprised by the novelty of the matter, I was standing a moment wordless, while the various discussions of the anatomists so rich in insinuations and not less in words . . . . . came to my mind . . . ." "Preparing myself for the experiment I grasped a very sharp scalpel and made an incision into one of these bundles. Scarcely had I touched it, when I saw a fluid like milk or cream gush forth. Noticing this, I hardly succeeded in repressing my joy: addressing myself to the bystanders, I exclaimed as Archimedes 'Eureka!' and invited them to observe the interesting play of this unusual phenomenon."




*Fig. 2.* Asellius (1581—1626), the discoverer of the lymphatic system

It is characteristic of the perspicacity of Asellius and the correctness of his conclusions that, when on the following day he performed vivisection on another dog and failed to discover the said phenomenon, he at once thought that his failure might be due to the fact that this animal — in contrast to that of the preceding day — had been dissected on an empty stomach. He dissected the third animal a few hours after an abundant meal and was once more able to observe the mesen-

teric lymphatic vessels as in the first case. He demonstrated them hereafter in various mammals, such as cats, sheep, lambs, cows, goats, horses, etc.

Chyle vessels in man were first demonstrated by Pecquet (1651) on the corpse of an executed criminal. Pecquet described also the thoracic duct and the Cisterna chyli. In 1647, as a student at Montpellier, he was present at the autopsy of a dog: when the heart was isolated he saw a white fluid pour forth into the thoracic cavity, which he took first for pus. After a careful preparation, however, he found the thoracic duct. Almost simultaneously with Pecquet the thoracic duct was described by Van Horne (1652), a professor of anatomy at Leyden. According to Zhdanov (1949), Vesalius had discovered the thoracic duct before Pecquet: he, however, failed to recognize its significance and termed it "Vena alba thoracis".



*Fig* 3. Picture of chyle vessels (Asellius 1627)

It is to Thomas Bartholinus and Olaus Rudbeck that credit is due for having assembled the accumulated pieces of mosaic and recognized the lymphatic system as such.

Rudbeck (1630—1708), a student at Uppsala and a disciple of Stefanius, studied in the winter of 1650/51 the chyle vessels which, since the discovery of Asellius, had been supposed to be the only pathway for food absorption from the intestines and were thought to run to the liver as the organ of blood production. In accordance with the medical opinion [illegible] tulate" that the [illegible] ing the portal [illegible] were dilated between the ligature and the liver and in a state of collapse beneath the ligature. At first he believed that these vessels conveyed pathological substances from the liver into the pancreas and that they were drained by the duct of Wirsung. At the same time, he discovered — first in the calf and then in the cat — the thoracic

duct, its inflow in the large veins, and also its connection with the Cisterna chyli. He recognized later the lymphatics of the oesophagus, colon, rectum and spermatic duct in dogs, sheep and other animals. Christine, the Queen of Sweden, keenly interested in scientific researches, happened to stay at Uppsala in April, 1652. At the request of the queen, Stenius delivered a lecture in the presence of the Court

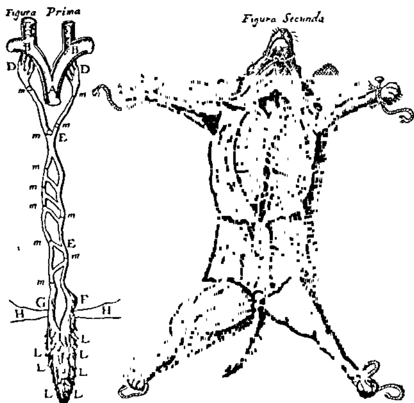



*Fig 4.* Thoracic duct and cisterna chyli in the work „Experimenta nova anatomica" of Pecquet, published in 1651

about the discoveries of Rudbeck who then demonstrated his discoveries on a dog. Besides the queen her court-physicians (Palmeron, Bromsius, Wullen) as well as numerous students of the university attended the demonstration. It was on this occasion that the physicians told Rudbeck that the thoracic duct had already been described by Pecquet, a fact hitherto unknown to him. In May, 1652, Rudbeck publicly defended his dissertation ("De circulatione sanguinis") in which he denied that the liver was in any way involved in haematopoiesis ("in concoctione sanguinis"). It was at this time that he prepared

his drawings of the thoracic duct and the lymph vessels discovered by him. *Rudbeck continued his researches and dissected not less than 400 animals*; relying on the evidence of these researches he was firmly convinced of the correctness of his observations. Preparing a study-tour abroad, he wrote down the results obtained by him up to that

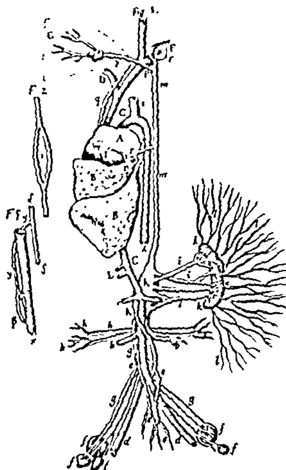


*Fig.* 5. Thoracic duct and principal lymph trunks (from the work "Nova exercitatio anatomica .." [1653] of Olaus Rudbeck)

time, and published them under the title "Nova exercitatio anatomica, exhibens ductus hepaticos aquosos et vasa glandularum serosa nunc primum inventa, aeneisque figuris delineata ab Olao Rudbeck sueco" (1653). In this brief work of 36 pages, he describes the lymphatic system (treating, however, of the hepatic lymph vessels separately). The descriptions are still astonishingly precise and exact. He describes

(and draws), for example, also the valves of the lymphatics which were forgotten later, to be rediscovered and described anew in detail by Ruyschius. In contrast to Bartholinus who described the lymph as a crystal-clear waterlike fluid, Rudbeck declares it to be salty and to coagulate like blood. Rudbeck expresses practically no opinion concerning the function of the lymph vascular system. Relying on the fact that chyle vessels do not empty into the liver, he contests the role of liver as a haematopoietic organ. In his opinion, the task of the liver consists in the production of bile and also in relieving the blood from superfluous water which flows then through the lymphatics into the Cisterna chyli. The other lymphatics would convey the lymph from the cavities of the body and the lymph nodes. He perceived the practical importance of this discovery in the fact that *ascites and oedema resulted from the occlusion of the lymphatic vessels.*



*Fig. 6* The human lymph vascular system (from the work "De lacteis thoracis.." [1652] of Thomas Bartholinus)

Rudbeck's work appeared in the winter of 1653. After this, he went on a study-tour. Arriving at Hamburg, he became acquainted with the treatise of Bartholinus on lymphatic vessels published in May of the same year.

In 1647, Thomas Bartholinus was appointed professor of mathematics in Copenhagen, and one year later he accepted the chair of anatomy, a post that seemed more convenient to him It was after the discovery of Pecquet that, in 1652, he began to concern himself with the anatomy of the thoracic duct and the chyle vessels. He noticed that the lymphatic vessels running to the liver were not chyle vessels and not emptying themselves in the liver but emerging from it. He regarded his observation at that time as an exception ("lusus naturae") since it was in contradiction to the contemporary concept concerning the function of the liver. Although — as can be seen from a letter written to his friend Arnisaeus — a few months later he began to have certain doubts as to the haematopoietic role of the liver, in his

work "De lacteis thoracis", issued in May, 1652, he was still eager to prove that the chyle vessels ran to the liver and that the liver was the haematopoietic organ "par excellence". Subsequent investigations convinced him, however, of the incorrectness of this theory and it was in these circumstances that his paper: "Vasa lymphatica nuper Hafniae inventa", dated 1st May, 1653, was published. Drawing the proper conclusions from his observations he ceased to consider the liver as a centre of blood production; he went so far as to write a solemn rhymed epitaph of the liver, this "princeps" of the body; this poem is absolutely peerless in medical literature, and so it seems worth while to quote it here:

"Siste Viator /Clauditur hoc tumulo qui tumulavit /plurimos/ princeps corporis tui cocus et /arbiter/ Hepar notum seculis/sed/ ignotum naturae /quod/ nominis majestatem et dignitatis /fama firmavit/ Tamdiu coxit/ donec cum cruento imperio seipsum /decoxerit/ Abi sine jecore viator/ Bilemque hepati concede/ ut sine bile bene /tibi coquas/ illi preceris."

What then happened is not too edifying. A very violent dispute regarding the question of priority flared up between Rudbeck and Bartholinus; its tone, as was the custom in those times, was far from distinguished. At first, Bartholinus did not participate in this debate personally and replied to Rudbeck only through his pupil Bogdanus (1654). Apart from reciprocal invective, they accused one another of plagiarism. In this second edition of his work, Bartholinus published data to prove that he had recognized the liver and the peripheral lymphatics before Rudbeck, but it is undoubted that Rudbeck was the first to discover their interconnection and to describe the lymph vascular system as a whole. From this point of view it is only of minor importance that Rudbeck's book was published a few months later than that of Bartholinus: he had demonstrated the observations described in it already much earlier before the whole university, in the presence of the court and of foreign physicians which, according to the custom of the age, was equivalent to publication. Besides, Rudbeck's knowledge was much more profound owing to his many systematic researches and also his illustrations more precise, so that he can by no means be regarded as guilty of plagiarism. This accusation was repeatedly made against Bartholinus: it was even alleged that a German physician or student, present at Rudbeck's famous demonstration, had noted the happenings and communicated them to Bartholinus, who hurriedly published them. In distinction from Tigerstedt (1893), who takes no sides in this question but restricts himself to the mere recording of facts, we agree with Bartels (1909) who regards young Rudbeck's priority as incontestable.

We have dealt somewhat lengthily with the question of the discovery of the lymph vascular apparatus as a fluid-conveying system which exists side by side with the arterial and venous systems, so as to give a good idea of the anatomical and physiological knowledge and

concepts of that age. If we take them into account we cannot be surprised that the theory of lymph circulation and lymphatic system had so many opponents. The correctness of Rudbeck's view, illustrated schematically in his first work, was acknowledged only after a century, in connection with the works of Hunter (1762). A part of the false views goes back to Bartholinus and his school, who, because of the milk-like white colour of the chyle, assumed the existence of a correlation between the chyle vessels and the mammary glands as well as the uterus. They took the newly discovered pancreatic duct for a lymph vessel and presumed that it conveyed the chyle from the duodenum to the pancreas, from which it was supposed to flow through the lymphatics. This theory was based on the fact that the mesenteric lymph nodes — which form a large bundle in certain animals — were not distinguished from the pancreas and designated by the common term Pancreas Aselli. It seems superfluous to enumerate all other theories of a still more fantastic nature. It is only natural that they encountered justified scepticism on the part of the greatest medical figures of the age, e.g. W. Harvey.

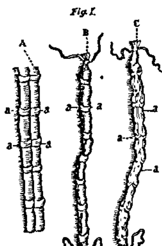



*Fig. 7.* Valves in the lymphatics (according to F. Ruyschius Opera omnia, 1721)

It was the progress of methodology which played an important part in the further development of the knowledge concerning the lymphatic system. A new method in the history of these researches was that of mercury injection, a discovery of Anton Nuck (1692) which made it possible to examine preparations in their connection and to preserve the preparations so injected. This technique of injection facilitated the fundamental works of Cruikshank (1789) and Mascagni (1787) on the topography of lymph vessels; both investigators injected the lymphatics in all parts of the human body, and their wonderful illustrations are still appropriate.

Hence, by the end of the 18th century, all anatomical researches with regard to lymph vessels and lymph trunks of major importance had, on the whole, been terminated, whilst the origin of lymph vessels, lymph capillaries and small lymphatics inside of the organs was still unknown.

There existed only nebulous ideas also about the origin of lymph. It was, for instance, assumed generally right up to the forties of the last century that the lymph passed from the blood stream into the lymphatics and that the latter were directly communicating with the blood vessels through a very fine and thin tubular system, the Vasa

serosa. This theory was abandoned only when Schwann discovered the cell as th ... ism. Virchow, in his " ... ence of Vasa serosa, l ... for — in his opinion ... etween circulatory and lymhatic system trough intracellular canaliculi. The next step followed when v. Recklingshausen (1863) denied a direct connection between the systems; he suggested that the end of the smallest lymph channels, the lymph capillaries, was open and the fluid passed through these open endings or other apertures, the *stomata*, from the interstitial space into the lymphatics. It was probably His (1863) who first claimed that the lymph vascular apparatus formed a closed system of tubes, a fact that could not be proved in a satisfactory manner until much later. The problem of the lymphatics in the different organs and capillary regions was still far from being elucidated. The investigations if Russian authors were extremely valuable in this connection, e. g. Afanasiew (The lymphatics of serous cavities, 1868); Eberth and Belajeff (The lymphatics of the heart, 1866); Vissotski (The lymphatics of the aponeuroses, 1877); Dogiel (1880, 1883); Savarykin (1863); etc.

The question regarding the origin of lymph was only elucidated much later; discussions about this question were continued for more than half a century and did not really come to a close until the end of the last century. As has been pointed out, the first notions in this respect were extremely nebulous. Rudbeck, but also Bartholinus, distinguished liver lymph and chyle. They took the latter for food absorbed from the intestines and regarded the first as a fluid filtered from the blood. Van Horne, otherwise an excellent expert of the anatomy of lymphatics, claimed that chyle was transformed into vapour in the lymph channels as a consequence of heat and gained access to the circulation in this condition. Until the middle of the last century, i.e. as long as a direct connection between blood and lymph vessels was assumed, the theory that the fluid passed through the thin Vasa serosa from the blood capillaries into the lymphatics seemed to offer a good explanation of the origin of lymph. Later, however, it was Ludwig's and Paschutin's theory of filtration which had to contend with Heidenhain's theory of secretion. By the last quarter of the past century, Ludwig's theory of filtration had come to be regarded as obsolete, and Heidenhain could explain the action of his "lymphagoga" of the 1st and 2nd order only by assuming that lymph was actively secreted by the blood capillaries. For the sake of completeness let us mention also Asher's and Barbera's theories according to which lymph takes its origin in the organs as a result of their function. After the reign of vitalism during several decades, it was Starling who demonstrated the erroneousness of Heidenhain's theory by very fine experiments; he proved filtration from blood capillaries to be a decisive factor in the genesis of lymph and/or interstitial fluid. We do not propose to expa-

tiate upon this subject at this point: this, as also the role of lymphatics in the absorption of fluids will be treated in detail in subsequent chapters.

This historical review would be incomplete if we omitted to speak of the importance that pathologists used to attribute to the diseases of the lymphatic system, i.e. if we did not dwell briefly on the progress of our knowledge about disturbances of the lymphatic system. We must admit that our knowledge in this respect is still very scarce.

Rudbeck and Bartholinus, the two discoverers of the lymphatic system, ascribed, as we have seen, the origin of oedema and ascites to the occlusion of the lymphatics. The idea that the retention of lymph or chyle is due to a mechanical interruption of the lymph paths appears to be the simplest, the most evident, and is as a matter of fact the oldest theory. It prevailed in the 17th and 18th centuries. Lymph, "the nutrient succus", was the "Fons aegritudinum" (Mascagni) and it was a general tendency to ascribe as many diseases as possible to "contaminated" and "stagnant" lymph Every tumescence of the lymph nodes, e. g. leukemia, tumours and tuberculosis of lymphatic nodes, lymphogranulomatosis etc. was attributed to this "bad nutrient succus". Adventurous and speculative ideas found a fertile soil in the scanty physiological and pathological knowledge of that age. Elephantiasis was also counted among the diseases of the lymphatic system although evidently no clear-cut line was drawn between real elephantiasis, and cardiac oedema, or different dermatological diseases associated with a thickening of the skin (e.g. lepra tuberosa).

Although Cruikshank (1789), in his famous book, already considers with a critical eye the numerous absurdities to be found in the medical literature of his age, pathologists were still very far from solving the problem of the occlusion of the lymph paths and its consequences. While as far back as 1774 Hewson suggested that a cicatrization of lymph nodes would give rise to collaterals for the removal of congested lymph, the question whether an obliteration of the lymph nodes had any pathological significance and if so in what such significance consisted remained controversial for a long time.

Early literature was compiled (true, with not too much criticism) by Gross and published in 1914/1916 in a series of publications each of which was nearly the size of a monograph. Numerous cases of diseases are cited therein which the authors thought were due to the obstruction of or a diseased condition of the lymphatics and lymph nodes. This old, very rich collection of case histories cannot and must not be judged too critically. The descriptions are deficient, unclear and reflect the medical errors and false notions of their epoch. The contradictory descriptions do not enable us to form any uniform picture of the notions of that age concerning the diseases of the lymphatic system: they would, at the most, allow us to quote a few curiosities and this seems to us to be superfluous.

# CHAPTER II

## PHYLOGENESIS OF LYMPHATICS

A lymphatic system is encountered only in vertebrate animals, and even here only among those of a higher organization: in the lower classes, where the circulatory system is scarcely differentiated no independent lymphatic apparatus exists. Lower vertebrates and invertebrates still possess a uniform haemolymphatic system. This is to say that the venous system performs also the functions of the lymphatic apparatus. We are referring in the first place to the fact that, in the absence of a chyle vascular apparatus, the absorption of nutritive substances from the gastro-intestinal tract is performed by the veins. For instance, we cannot, in the strict sense of the word, speak of a lymphatic system in the Leptocardii or in the Cyclostomata. The sinal system of Cyclostomata, as described in detail by Tretjakoff (1926—1930), is undoubtedly suggestive of the lymphatic sinuses known in certain other animals; these sinuses contain, however, blood and are in direct connection with the blood-vessel system. We must therefore conclude (for detailed literature see Weidenreich 1931/1933) that a still undifferentiated haemolymphatic system exists also in the Cyclostomata.

It is in *teleosts* that the differentiation of a lymphatic system is first observable. A comparison of the circulatory apparatus between Chondrichthyses (cartilaginous fishes) and Telostei (teleosts) is highly instructive in respect of the phylogenesis of lymph vessels: the vessels which, in the teleosts, belong to the lymphatic system exist also in the cartilaginous fish but contain blood and so belong to the venous system. Among the cartilaginous fishes only certain torpedoes *(Torpedo marmorata, Torpedo ocellata)* constitute an exception. In these, the visceral system (i. e. the chyle vessels) contains blood no longer and has, thus, differentiated itself from the venous system. What we find in cartilaginous fishes is already a more or less complete separation of the two parts of the lymph vascular apparatus, i.e. the visceral system of the gastro-intestinal tract and the parietal system. The lymph vessels belonging to the visceral system form a profuse network on the surface of the organs covered with a serous membrane, and unite in the Vas lymphatic intestino-mesentericum. Besides the longitudinal superficial lymphatic vessels *(Vas superficiale dorsale, ventrale, laterale)*, to the parietal system belong also the deeper lymph cavities, the lymph sinuses. Of the deeper, longitudinally coursing lymphatics we want to note the spinal and subvertebral trunks. Hoyer and Michalski (1922) regard the latter as being identical with the thoracic duct of higher vertebrates. The lymph sinuses are,

weighing 90 Kg. After an equilibration period of two hours, a blood sample is drawn and the plasma separated and analyzed. The $D_2O$[1] concentration is found to be 0.200 volumes per cent of the plasma water (0.2 ml. of $D_2O$ per 100 ml. of plasma water). During a two hour equilibration period, urinary, respiratory, and cutaneous losses have been found to average 0.4 per cent of the administered dose. Substituting in the equation given above:

$$\text{Volume of Distribution} = \frac{(100 - 0.4) \times 100}{0.200} = 49{,}800 \text{ ml. or } 49.8 \text{ liters}$$

Since our subject weighed 90 Kg., body water constitutes $\frac{49.8}{90} \times 100 = 55.3$ per cent of body weight. Although the techniques for quantifying $D_2O$ and $T_2O$ are remarkably precise, with errors of $\pm$ 1.0 per cent, the overall accuracy of the measurement of total body water is appreciably less. Thus deuterium exchanges with certain labile hydrogen atoms of proteins and carbohydrates, the net effect of which is to increase the apparent volume of distribution of $D_2O$ by one to three per cent of body weight above its true value. Although the measurement of total body water by the isotope dilution method is relatively precise, the possible error is a liter or more. Accordingly, changes in body water over short periods of time can be estimated more precisely from changes in body weight than from changes in volume of $D_2O$ distribution.

*Plasma Volume* may be estimated by measuring the volume of distribution of materials which are largely retained within the vascular compartment when they are injected intravenously. The dye Evans blue binds firmly to the plasma proteins, hence is retained within the vascular system to whatever extent the proteins are retained. Lymph from muscle contains a small amount of protein, whereas that from the liver is rich in protein. Accordingly, Evans blue is found in low concentration in muscle lymph and in much higher concentration in liver lymph. The volume of distribution of the dye, calculated by means of the equation given for total body water, somewhat overestimates plasma volume, for it is impossible to measure the amount of dye present in the lymph of

[1]$D_2O$ when mixed with body water becomes largely DHO. It simplifies the calculation of volume of distribution and introduces no error to consider it as $D_2O$

advanced disease, composition as well as volume may be altered. *Early or in mild disease, edema collects in dependent parts of the body.* Later it becomes generalized. Two kilograms, or about 5 lb. of excess interstitial fluid is the least that can be recognized with certainty by the "pitting" which occurs on pressure over the tibia. In its most severe form represented by anasarca and/or massive ascites, edema fluid may collect to the extent of 50 lb. or more. Strictly speaking, ascites represents an expansion of the peritoneal moiety of transcellular fluid. The composition of ascitic fluid is not essentially different from that of edema fluid except for its high protein content. It is no doubt a transudate of intrahepatic and portal capillaries, modified to some extent by the peritoneal epithelium. Relative isolation from lymphatic and vascular channels makes its mobilization more difficult. However, for the purposes of our discussion, ascitic fluid and edema fluid may be considered together as biochemical and functional equivalents.

*Disturbances of Function in Edema.* Tissue functions are disturbed by edema in no less than two ways. First, an excess of interstitial fluid, rendering tissues boggy and turgid, interferes mechanically with their functions. For example, digestive disturbances, which are commonly associated with edema, are presumably due in part to altered gut motility. The most common complaints of edematous patients are related to swelling of the ankles, protuberance of the abdomen, and gain in weight, all of which have their adverse mechanical as well as cosmetic implications. Second, an excess of interstitial fluid constitutes a stagnant pool interposed between circulating blood plasma and tissue cells. Diffusion distances are increased and, as a consequence, the concentrations of nutriments are lower and of wastes higher within and immediately surrounding cells. Because the environment is less favorable, cell function may be disturbed. Pulmonary edema constitutes a special threat in that it reduces lung compliance, interferes with ventilation of the alveoli, and serves as a barrier to exchange of gases between alveolar spaces and blood.

(6) It should be readily determinable in low concentration in plasma with a high degree of precision. Needless to say, no such substance is known at the present time. However, were one to exist, its volume of distribution, calculated from the general equation given for total body water, would give a precise measure of extracellular volume.

It is the author's opinion that radiosulfur-35 as sulfate ion exhibits more of the ideal characteristics outlined above than any other of the several substances proposed. In nephrectomized dogs where one can eliminate the disturbing element of renal excretion, Swan *et al.* have shown that radiosulfate, mannitol, and thiosulfate are distributed in equal volumes, averaging about 22 per cent of body weight. In the presence of normal renal function, rapid excretion of both mannitol and thiosulfate render measurements of extracellular volume by direct dilution methods suspect. Radiosulfate is much more slowly excreted and less rapidly metabolized than thiosulfate and mannitol, hence preferable for measurement of extracellular volume.

It is possible to avoid the complication of high rate of excretion of mannitol, thiosulfate, and other substances whose volumes of distribution have been proposed as measures of extracellular fluid in the following way. The substance is infused intravenously at a constant rate until contancy of plasma concentration is achieved and until diffusion equilibrium between plasma and interstitial fluid is attained. Two to six hours or more may be required. At this time a blood sample is drawn and the concentration of the substance in plasma water is determined. The bladder is emptied and the infusion stopped. All urine is collected until the excretion of the substance in question is complete. The total quantity excreted, divided by the concentration in plasma water at the time the infusion was stopped, gives the apparent volume of distribution. The method is laborious, time consuming and subject to errors which render it no more accurate than the simpler radiosulfate method.

*Changes in Interstitial Volume in Edema.* Edema represents an abnormal accumulation of interstitial fluid. In its more common form, the ionic composition and osmotic pressure of this fluid are essentially normal, only volume is increased. However, in more

*Chapter II*

# IONIC COMPOSITION OF BODY FLUIDS

IN discussing the composition of the body fluids, it is necessary to utilize the terminology of chemical equivalents,[*] familiar enough to recent graduates of medicine, but perhaps sufficiently unfamiliar to the more mature to justify brief explanation. When the concentration of each ionic constituent of a complex solution like interstitial fluid is expressed in milliequivalents (mEq.) per liter, the sum of the concentrations of all the positive ions (cations) such as sodium, potassium, calcium, and magnesium exactly equals the sum of the concentrations of all the negative ions (anions) such as chloride, bicarbonate, sulfate, phosphate and protein. Balance in terms of equivalents is obligatory, for solutions must be electricallly neutral; each positive ionic charge must be balanced by a negative ionic charge. Balance in terms of weights per unit volume (milligram per cent) is impossible to achieve.

*Definition of Chemical Equivalents.* A milliequivalent (mEq.) is 1/1000th of an equivalent. Ion concentrations in body fluids, when expressed in mEq., are whole numbers, hence are more convenient to handle than if expressed in equivalents. One milligram

[*]On a trip to Scotland some years ago, my wife and I stopped at a small hotel in the highlands. Leaving early, I anticipated the usual delay in toting up the bill for bar, dinner, bed and breakfast. Instead, the desk clerk, after a few preliminary scratches, promptly quoted to the exact penny the figure I had laborously worked out earlier that morning On inquiry as to the method employed, he stated that it was really quite simple "I convert pounds and shillings to dollars, add up the bill in dollars and reconvert to pounds and shillings." Treating chemical composition in any terms other than that of equivalents involves a system of conversions, calculations, and reconversions considerably more formidable than those required by the Scottish currency

## SUMMARY

Water constitutes some 50 to 60 per cent of the weight of the normal human body. This water is distributed among a plasma compartment (4 per cent), an interstitial compartment (16 per cent), a transcellular compartment (1 to 6 per cent), and a cellular compartment (30 to 35 per cent) of body weight. Edema represents primarily an abnormal expansion of volume of interstitial fluid; composition may be entirely normal. Edema interferes mechanically with tissue functions and hinders exchange of nutriments and wastes between blood and tissue cells. In the lung, edema reduces pulmonary compliance, interferes with alveolar ventilation and restricts diffusion of gases between blood and alveolar spaces.

## REFERENCES

1. MANERY, J.: Water and electrolyte metabolism. *Physiol. Rev.*, *34:* 334, 1954.
2. SCHLOERB, P. R., FRIIS-HANSEN, B. J., EDELMAN, I. S., SOLOMON, A., and MOORE, F. D.: The measurement of total body water in the human subject by deuterium oxide dilution with a consideration of the dynamics of deuterium distribution. *J. Clin. Invest.*, *29:*1296, 1950.
3. SCHULTZ, A. L., HAMMERSTEN, J. F., HELLER, B. I. and EBERT, R. V.: A critical comparison of the $T_{1824}$ dye and iodinated albumin methods for plasma volume measurement. *J. Clin. Invest.*, *32:*107, 1953.
4. STRAUSS, M. B.: *Body Water in Man.* Boston, Little, Brown & Co., 1957.
5. SWAN, R. C., MADISSO, H., and PITTS, R. F: Measurement of extracellular fluid volume in nephrectomized dogs. *J. Clin. Invest.*, *33:*1447, 1954.

stitial fluid and plasma are real, it is apparent from Table I that they are not large. As a first approximation, they can be neglected and interstitial fluid can be assumed to have the ionic structure of plasma.

## IONIC CONSTITUTION OF INTRACELLULAR FLUID

Owing to several difficulties, knowledge of the ionic composition of intracellular fluid is far less exact than is that of extracellular fluid. It is impossible to analyze cell contents directly. Tissues must be analyzed and cell composition calculated from total minus extracellular ionic concentrations. Since the volume of the extracellular phase of tissues cannot be determined with any degree of certainty, both intracellular volume and composition can only be approximated. Furthermore, the various tissues of the body differ in their protein, lipid and water contents and no doubt in their ionic constitutions as well. One cannot, therefore, describe a general chemical structure of intracellular fluid applicable to all cells. Finally, one cannot determine the physical state within the living cell of some of the electrolytes, whether bound or free, whether ionized or un-ionized.

Certain features which are characteristic of intracellular fluids and which serve to distinguish them from extracellular fluids are evident in the observations on skeletal muscle summarized in Table II. The major intracellular cations are potassium and magnesium. Relatively little sodium, the major extracellular cation, exists in

TABLE II

IONIC STRUCTURE OF MUSCLE

| | *mEq/L.* $H_2O$ |
|---|---|
| Na | 10 |
| K | 160 |
| Mg | 35 |
| Cl | 2 |
| Protein | 55 |
| $PO_4$ (organic) | 140 |

TABLE I
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| | *Plasma* | *Plasma Water* | *Interstitial Fluid* |
|---|---|---|---|
| | *(mEq./L.*)* | *(mEq /L.*)* | *(mEq /L water†)* |
| Na | 142 | 151 | 144 |
| K | 4 | 4.3 | 4.0 |
| Ca | 5 | 5.4 | 2.5 |
| Mg | 3 | 3.2 | 1.5 |
| ΣCation | 154 | 163.9 | 152.0 |
| Cl | 103 | 109.7 | 114 |
| $HCO_3$ | 27 | 28.7 | 30 |
| $PO_4$ | 2 | 2.1 | 2.0 |
| $SO_4$ | 1 | 1.1 | 1.0 |
| Organic Acid | 5 | 5.3 | 5.0 |
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present in somewhat greater concentrations to take their place. (b) The sum of all cations must equal the sum of all anions both in interstitial fluid and in plasma. (c) The osmotic pressure (dependent on numbers of solute particles) of interstitial fluid must equal that of plasma, except for the slight colloid osmotic (oncotic) force due to the presence of proteins in plasma. This oncotic force is balanced by a hydrostatic force, the capillary blood pressure. As is evident from Table I, the diffusible (non-protein) anions of interstitial fluid are slightly more concentrated than are those of plasma water. In contrast the diffusible cations are slightly less concentrated than are those of plasma water. Calcium and magnesium are considerably lower in concentration in interstitial fluid because they are in part bound to proteins of the plasma, hence are non diffusible. Although the differences in ionic composition of inter-

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reactivate the cell pumps and restore normal cellular and plasma concentrations.

Figure 3 A and B illustrate the general concepts of Glynn, Shaw, Hodgkin, Keynes and others of the ion pump of erythrocytes, muscle cells and nerve cells. The passive movements of sodium and potassium into and out of cells are shown by the straight arrows to either side of Figure 3A. The passive diffusion of sodium into the cell (sodium influx, heavy arrow) exceeds the passive diffusion out of cell (sodium efflux) for it occurs downhill along a large concentration gradient. In contrast, passive efflux of potassium exceeds passive influx for the concentration gradient is the reverse of that for sodium. Were passive influx of sodium and passive efflux of potassium to continue unopposed, concentrations of the two ion species inside and outside the cell would equalize. The curved arrows connected by a circle represent the active fluxes which oppose the attainment of diffusion equilibrium. Sodium is pumped out of the cell, potassium is pumped into the cell, and the two processes seem to be linked. But little is known of the energetics of this coupled transport system, other than the fact that it utilizes phosphate bond energy.

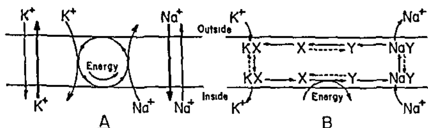



Fig 3. Mechanisms of transport of sodium and potassium across cell membranes. A. Straight arrows, passive diffusion of ions. Curved arrows, active transport of ions. B. Hypothetical coupled carrier mechanism which might actively transport potassium into cells and eject sodium from cells (From I. M Glynn. *J Physiol.* 134:278, 1956.)

Cell membranes in general are polarized. negative inside, positive outside, and this polarization seems in many instances to be related to the difference in potassium concentration on the two sides of the membrane. The potential difference (P.D.) across many mam-

muscle cells. The major intracellular anions are organic phosphates (adenosine mono-, di-, and triphosphates, glycerophosphate, creatine phosphate) and proteins. Relatively little chloride, the major extracellular anion, exisits in muscle cells.[7] Although muscle has been used to illustrate the marked differences in composition which distinguish intracellular from extracellular fluids, other cell types exhibit differences which are qualitatively similar.

The total osmolal concentration of intracellular fluid is thought to be essentially the same as that of extracellular fluid.[8] However, this fact cannot be derived from the data given in Tables I and II, for the osmotic activity of the polyvalent ions, magnesium, organic phosphates and protein, is unknown. It is even impossible to balance total cations against total anions for the concentrations of a number of components are uncertain. However, exact ionic balance must exist.

*Maintenance of Differences in Ionic Composition.* The marked differences in the concentrations of sodium, potassium, and chloride between cell contents and their surrounding fluid environment do not derive from any absolute ionic impermeability of cell membranes. The radioisotopes of these ions, introduced into extracellular fluid, exchange more or less rapidly with their non-radioactive counterparts within cells. One is forced to conclude that the tendency of sodium ions to diffuse into cells and of potassium ions to diffuse out along their concentration gradients must be counteracted by the active outward pumping of sodium ions with or without the active inward pumping of potassium ions. It is probable that transport of both ions is active.

These ion pumps are metabolically activated. Cold and certain metabolic inhibitors inactivate the pumps. The most familiar example of the changes in cell composition which occur when the ion pumps fail is the loss of red blood cell potassium to plasma and the entry of plasma sodium into red blood cells when bank blood is stored in the cold. Rewarming the blood and adding glucose

[7]Certain cells contain more chloride than muscle, e.g., gastric mucosa, kidney, skin, testis, ovary and red blood cells.

[8]The view of Robinson, Opie and others that cells are hypertonic to their surroundings has not been generally accepted.

limiting value, the system will operate in counter clockwise direction, pumping sodium out of and potassium into the cell.

The distribution of chloride (low inside the muscle cell, high outside) is that predicted by the Gibbs-Donnan rule and is analogous to the distribution already described between plasma and interstitial fluid. The difference in chloride concentration across the cell membrane is, however, far greater than that across the capillary membrane, for the anions of the intracellular fluid are largely non-diffusible, whereas those of plasma, except for protein, are largely diffusible. The concentration of chloride within the cell must be low to satisfy the requirements that total anions must equal total cations and that total osmolal concentration of intracellular fluid must equal that of interstitial fluid. If the potassium content of cells is increased acutely as it is by the infusion of potassium salts, their chloride content increases. Conversely those cells in which the chloride concentration is normally high (gastric mucosa, gonad, etc.) must exhibit lower intracellular concentrations of non-diffusible anions.

Little is known concerning the bicarbonate ion content of muscle and most tissue cells, largely because of uncertainty in partitioning the carbon dioxide which can be extracted from them among the several forms in which it is known to exist: dissolved carbon dioxide and carbonic acid, carbamino bound carbon dioxide, and bicarbonate ion. Nearly all would accept the thesis that cells are freely permeable to dissolved carbon dioxide. If true, the concentration of dissolved carbon dioxide and of carbonic acid will be essentially the same in cell water and in the water of blood plasma and interstitial fluid, namely 1.2 to 1.4 mEq. per liter. Opinion varies as to the relative proportion of the remainder of the carbon dioxide (6 to 10 mEq. per liter) present as bicarbonate ion and in carbamino combination. If present largely in carbamino combination, bicarbonate ion concentration and cell pH will be low. If present largely in the form of bicarbonate, cell pH will approach neutrality. If cell bicarbonate concentration and pH are low ($\sim$pH 6), it is possible that the cell membrane is relatively permeable to bicarbonate ion and that low internal bicarbonate concentration is an expression of a Donnan ion distribution similar to

malian cell membranes can be shown to vary with the concentration of potassium in the interstitial fluid in the following manner:

$$\text{P.D (millivolts)} = 61 \times \log \frac{[^{K}\text{cellular}]}{[^{K}\text{interstitial}]}$$

The P.D. can be considered as a potassium diffusion potential, the origin of which is explained in the following manner. The cell membrane is absolutely impermeable to the large polyvalent protein and organic phosphate anions of the cell contents. The cell is effectively, although not actually impermeable to sodium by virtue of the operation of membrane pumps which continuously eject the sodium which diffuses in. In essence the cell membrane can be considered to be relatively permeable only to potassium ions and perhaps to chloride ions. Positively charged potassium ions tend to diffuse out of the cell, downhill along their concentration gradient. They are restrained by the increasing negative charge left within the cell. A state is reached at which the outward diffusion of potassium, driven by concentration difference, is just balanced by increasing cellular negativity, restraining further diffusion. This state is described by the equation given above. If cellular concentration of potassium were ten times interstitial concentration, the P.D. would be 61 mv. ($\log 10 = 1$). If cellular and interstitial concentrations were equal, the P.D. would be 0.0 mv. ($\log 1 = 0$). Values of P.D. for most mammalian cells vary from 60 to 90 mv., corresponding to a ratio of cellular/interstitial potassium concentration of 10/1 to 30/1.

Figure 3B illustrates a hypothetical cyclical carrier system advanced to explain the active coupled ion fluxes in red cells, muscle and nerve. It is postulated that $K^+$ and $Na^+$ cross the cell membrane in combination with the carriers X and Y; X is $K^+$ specific; Y is $Na^+$ specific. The complexes KX and NaY are presumed to be freely diffusible within the substance of the membrane and in equilibruim with $K^+$ and X and with $Na^+$ and Y, respectively, at the inner and outer surfaces of the membrane. At the inner surface of the membrane, X is converted to Y by the expenditure of phosphate bond energy. So long as energy is supplied, and so long as the concentrations of $Na^+$ inside and of $K^+$ outside are above some

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malian cell membranes can be shown to vary with the concentration of potassium in the interstitial fluid in the following manner:

$$\text{P.D (millivolts)} = 61 \times \log \frac{[K_{cellular}]}{[K_{interstitial}]}$$

The P.D. can be considered as a potassium diffusion potential, the origin of which is explained in the following manner. The cell membrane is absolutely impermeable to the large polyvalent protein and organic phosphate anions of the cell contents. The cell is effectively, although not actually impermeable to sodium by virtue of the operation of membrane pumps which continuously eject the sodium which diffuses in. In essence the cell membrane can be considered to be relatively permeable only to potassium ions and perhaps to chloride ions. Positively charged potassium ions tend to diffuse out of the cell, downhill along their concentration gradient. They are restrained by the increasing negative charge left within the cell. A state is reached at which the outward diffusion of potassium, driven by concentration difference, is just balanced by increasing cellular negativity, restraining further diffusion. This state is described by the equation given above. If cellular concentration of potassium were ten times interstitial concentration, the P.D. would be 61 mv. (log 10 = 1). If cellular and interstitial concentrations were equal, the P.D. would be 0.0 mv. (log 1 = 0). Values of P.D. for most mammalian cells vary from 60 to 90 mv., corresponding to a ratio of cellular/interstitial potassium concentration of 10/1 to 30/1.

Figure 3B illustrates a hypothetical cyclical carrier system advanced to explain the active coupled ion fluxes in red cells, muscle and nerve. It is postulated that $K^+$ and $Na^+$ cross the cell membrane in combination with the carriers X and Y; X is $K^+$ specific; Y is $Na^+$ specific. The complexes KX and NaY are presumed to be freely diffusible within the substance of the membrane and in equilibruim with $K^+$ and X and with $Na^+$ and Y, respectively, at the inner and outer surfaces of the membrane. At the inner surface of the membrane, X is converted to Y by the expenditure of phosphate bond energy. So long as energy is supplied, and so long as the concentrations of $Na^+$ inside and of $K^+$ outside are above some

limiting value, the system will operate in counter clockwise direction, pumping sodium out of and potassium into the cell.

The distribution of chloride (low inside the muscle cell, high outside) is that predicted by the Gibbs-Donnan rule and is analogous to the distribution already described between plasma and interstitial fluid. The difference in chloride concentration across the cell membrane is, however, far greater than that across the capillary membrane, for the anions of the intracellular fluid are largely non-diffusible, whereas those of plasma, except for protein, are largely diffusible. The concentration of chloride within the cell must be low to satisfy the requirements that total anions must equal total cations and that total osmolal concentration of intracellular fluid must equal that of interstitial fluid. If the potassium content of cells is increased acutely as it is by the infusion of potassium salts, their chloride content increases. Conversely those cells in which the chloride concentration is normally high (gastric mucosa, gonad, etc.) must exhibit lower intracellular concentrations of non-diffusible anions.

Little is known concerning the bicarbonate ion content of muscle and most tissue cells, largely because of uncertainty in partitioning the carbon dioxide which can be extracted from them among the several forms in which it is known to exist: dissolved carbon dioxide and carbonic acid, carbamino bound carbon dioxide, and bicarbonate ion. Nearly all would accept the thesis that cells are freely permeable to dissolved carbon dioxide. If true, the concentration of dissolved carbon dioxide and of carbonic acid will be essentially the same in cell water and in the water of blood plasma and interstitial fluid, namely 1.2 to 1.4 mEq. per liter. Opinion varies as to the relative proportion of the remainder of the carbon dioxide (6 to 10 mEq. per liter) present as bicarbonate ion and in carbamino combination. If present largely in carbamino combination, bicarbonate ion concentration and cell pH will be low. If present largely in the form of bicarbonate, cell pH will approach neutrality. If cell bicarbonate concentration and pH are low (~pH 6), it is possible that the cell membrane is relatively permeable to bicarbonate ion and that low internal bicarbonate concentration is an expression of a Donnan ion distribution similar to

that for chloride. If cell bicarbonate concentration and pH are relatively high (~pH 7), it is possible that the cell membrane is considerably less permeable to bicarbonate than to chloride ion or that there is some active mechanism which regulates cell pH.

## OSMOTIC PRESSURE AND DISTRIBUTION OF WATER

Osmotic forces are of such importance in determining the distribution of water among the several fluid compartments of the body that it is important to have a clear appreciation of their mode of operation.

When a solution and pure solvent are separated by a semipermeable membrane, the solvent passes into the solution by a process known as osmosis. The osmotic pressure is that hydrostatic pressure which must be applied to the solution to prevent this inward migration of solvent (see Fig. 4). Cell membranes in general are more or less permeable to water but effectively impermeable to many crystalloidal solutes such as sodium, chloride and bicarbonate, hence may be classified as basically semipermeable. By effective impermeability is meant extrusion of a substance from a cell as rapidly as it enters under electrical and chemical forces.

When red blood cells are placed in distilled water, water enters by osmosis and the cells swell and hemolyze. If the cells are placed in 0.9 per cent saline, they undergo no change in volume. The osmolal concentration of the saline exactly equals that of the cell contents, i.e., the two are isosmotic. Since no osmotic swelling or shrinkage occurs, the saline is said to be isotonic.

A 1.8 per cent solution of urea has the same osmolal concentration as a 0.9 per cent solution of sodium chloride, i.e., the solutions are isosmotic. However, when red cells are placed in 1.8 per cent urea, they swell and hemolyze exactly as they do in distilled water. Obviously, the two solutions do not have physiologically equivalent osmotic pressures. Although isosmotic, they are not isotonic. The reason for this minor dilemma is the following. Red cell membranes, like most other cell membranes, are nearly as permeable to urea as they are to water. Therefore urea exerts no osmotic effect when separated from cell contents by membranes permeable to it. A solution of urea is physiologically equivalent to distilled water.

Capillaries are relatively permeable not only to water but also

to the major crystalloids of the blood plasma. In fact they restrict only the diffusion of colloidal proteins and lipids. Hence only transient and minor osmotic forces are developed across capillary walls when solutions of higher or lower osmolal concentrations are introduced into the circulation.

An osmotic pressure is a virtual pressure rather than a real pressure. It exists only when solution and solvent or two solutions of differing osmolal concentrations are separated by a membrane permeable to solvent but not to solute. It is commonly stated that a concentrated solution has a higher osmotic pressure than a dilute solution. This statement is ambiguous for two reasons. First, as noted above, the osmotic pressure is that hydrostatic pressure which must be applied to a solution to prevent inward migration of solvent across a semipermeable membrane. No hydrostatic pressure exists in either solvent or solution in isolation. Second, the force involved is dependent on diffusion of solvent, not per se on the presence of solute except insofar as it determines the concentration of solvent in a solution.

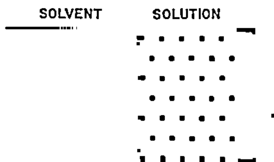



Fig 4. Kinetic formulation of osmotic pressure in terms of bidirectional diffusion of solvent across a membrane impermeable to solute.

One can formulate a kinetic picture of osmotic pressure in terms of the molecular motions of the solvent on the two sides of a cell membrane as illustrated in Figure 4. On the pure solvent side (or side of more dilute solution), water molecules in their unordered agitation strike against the membrane, and since it is permeable to water, some pass through. The same thing happens on the solution

side of the membrane with this difference; the water is somewhat diluted by the presence of the solute, so that its effective concentration, chemical potential, or escaping tendency is reduced. As a consequence, fewer water molecules per second pass from solution to solvent than in the reverse direction. The result is that there is a net flow of water from solvent to solution. The osmotic pressure is that hydrostatic pressure which must be applied to the solution to raise its chemical potential or escaping tendency to equal that of the pure solvent.

It is an unfailing source of surprise to the student when he is reminded of the osmotic forces developed when blood plasma, interstitial fluid, and cell contents are separated by semipermeable membranes from the pure solvent, water. These forces are of the order of magnitude of 6.7 atmospheres or 5,000 mm. Hg. His surprise is no doubt based on his greater familiarity with the more modest hydrostatic pressures which exist in the vascular system. The equality of osmolal concentrations of extracellular, transcellular and intracellular fluids results directly from the rapid distribution of water through capillary walls and cell membranes among all body fluid compartments, driven by what are potentially forces of great magnitude. The water distributes so that it has the same escaping tendency or chemical potential in each compartment.

The osmotic force which develops across a semipermeable membrane separating pure solvent from solution is dependent on the number of particles of solute per unit volume of solution. One gram molecular weight of glucose, namely 180 gm., containing $6.06 \times 10^{23}$ particles, is termed 1.0 osmol. One osmol of glucose dissolved in 22.4 liters of water depresses the chemical potential of the water by 1.0 atmosphere; i.e., a hydrostatic pressure of this magnitude must be applied to raise the chemical potential of the water in the solution to equal that of pure water. Or conversely 1.0 osmol of glucose in 1.0 liter of water depresses its chemical potential by the equivalent of 22.4 atmospheres. The similarity between the osmotic effect of a solute in solution and the pressure it would exert if it were a gas confined in the same volume is evident. In general the laws of osmotic pressure are similar to the gas laws. One gram molecular weight of sodium chloride, namely

58.5 gm., containing $6.06 \times 10^{23}$ molecules, dissociates into twice the number of ions in solution. Therefore 1.0 mol of sodium chloride exerts an osmotic effect equivalent to 2.0 osmols.[a] Calcium chloride, $CaCl_2$, dissociates into three particles; hence one gram molecular weight (111 gm.) exerts an osmotic effect of roughly 3.0 osmols.

The concentrations of the osmotically active components of the body fluids are commonly expressed in milliosmols (mOsm.) per liter or per Kg. of water; 1.0 mOsm. equalling 1/1000 osmol. One mOsm. of any solute, consisting of $6.06 \times 10^{20}$ particles dissolved in a liter of water exerts an osmotic effect equivalent to 17 mm. Hg. The osmotic activity of an electrolyte solution is dependent solely on numbers of particles, not on their charge. Accordingly, 1.0 mOsm. of univalent ions, Na, K, Cl, $HCO_3$ consists of the same number of particles as 1.0 mOsm. of divalent ions, Ca, Mg, $SO_4$. One mOsm. of univalent ions is 1.0 mEq.; 1.0 mOsm. of divalent ions is 2.0 mEq. Plasma, the transcellular fluids, interstitial fluid and cellular fluids have osmolal concentrations of roughly 300 mOsm per liter of water. Therefore the osmotic effect of the solutes contained in these fluids is equivalent to $17 \times 300 = 5100$ mm. Hg. Between 90 and 95 per cent of the osmotic activity of the solutes of plasma and interstitial fluid may be assigned to sodium, chloride and bicarbonate ions. Other ions and organic compounds such as glucose, amino acids and urea account for the remaining 5 to 10 per cent.

## COLLOID OSMOTIC PRESSURE AND DISTRIBUTION OF FLUID

The plasma proteins are substances of very high molecular weight· albumin, 69,000, globulins, 90,000 to over 1,000,000. Therefore, even though they are present in plasma in high concentration, 60 to 70 gm. per liter, they exert only small osmotic effects, equivalent on an average to a pressure of 28 mm. Hg. When one compares this protein or colloid osmotic effect with the total crys-

[a]Actually less, for the activity of a sodium chloride solution of finite concentration is not 1.0, i.e., the sodium and chloride ions interact and behave as though there were slightly less than two ions per molecule.

talloidal osmotic effect of 5100 mm. Hg, it seems rather insignificant. However, it has a physiological significance out of all proportion to its quantitative magnitude. As was pointed out above, the capillary walls are permeable not only to water, but also to all those crystalloidal solutes which account for the major fraction of the osmotic effect of the plasma and interstitial fluid. Accordingly, the crystalloids exert no osmotic effect across the capillary wall. In contrast the capillaries are relatively impermeable to protein and the protein concentration of lymph from most organs other than the liver is low. As a consequence, the plasma proteins exert nearly their full osmotic effect across the capillary membrane and oppose the filtration of fluid from capillary lumen to tissue interstices under the head of hydrostatic pressure which exists in the terminal vascular bed. Because albumin is the most abundant of the plasma proteins and has the lowest molecular weight, it exerts the major fraction of the colloid osmotic effect.

## TOTAL BODY STORES OF IONS

*Sodium.* The total body sodium of a normal adult male averages 60 mEq. per Kg. of body weight. A 70 Kg. man, therefore, contains in all 4200 mEq., or nearly 100 gm., of sodium. Bone, which constitutes only 15 per cent of body weight, contains from 40 to 45 per cent of the total sodium store, namely 1800 mEq. Of the 2400 mEq. of non-bone sodium, between 2000 and 2200 mEq. are present in the extracellular fluid. In round figures, 50 per cent of body sodium is extracellular, 40 per cent is associated with bone and 10 per cent is intracellular.

A more useful breakdown of sodium stores is into exchangeable and non-exchangeable moieties. Exchangeable sodium, measured by dilution of the radioactive isotopes $Na^{22}$ or $Na^{24}$, amounts to 42 mEq. per Kg. of body weight. This fraction includes all of extracellular sodium, all of intracellular sodium and somewhat less than half of bone sodium. The non-exchangeable fraction, amounting to 18.0 mEq. per Kg. of body weight, is largely associated with bone. Exchangeable sodium is of interest in that it is in diffusion equilibrium with plasma sodium. If sodium is lost from blood plasma into urine or into feces (diarrhea), that of the labile exchangeable

reservoir is available to reduce the fall in concentration when body water is restored. If sodium is retained in the body, as it is in developing edema, it is distributed into this labile exchangeable reservoir. The ion content of this reservoir can be measured in man by the technique of isotope dilution. While scarcely a bedside procedure, it is possible to employ this method to quantify precisely the extent of sodium retention or depletion (see below for method). Non-exchangeable sodium probably represents that adsorbed on the surfaces of the apatite crystals of bone, more specifically onto surfaces which are buried in the bone structure and completely isolated from blood plasma and interstitial fluid.

***Potassium.*** The total body potassium of a normal adult male averages 45 mEq. per Kg. of body weight. A 70 Kg. man, therefore contains in all 3150 mEq. or about 120 gm. of potassium. This potassium is almost entirely intracellular, only 60 mEq. or about 2 per cent is distributed in the extracellular fluid. Essentially all of body potassium is labile and exchangeable. As is evident from Table I, the concentration of potassium in blood plasma and interstitial fluid is low, some 4 mEq. per liter. Unfortunately, the plasma concentration of potassium is a very poor indicator of tissue potassium stores. In acute renal failure the discharge of a very small proportion of the large store of intracellular potassium will cause the extracellular concentration to rise to dangerous and possibly lethal levels of 8 to 10 mEq. per liter. On the other hand, in diabetic acidosis with normal renal function, a significant fraction of cellular potassium may be discharged into the extracellular fluid and excreted in the urine with little disturbance in plasma concentration. In chronic potassium depletion produced by prolonged vomiting or diarrhea, or by intensive diuretic therapy, sodium may replace an appreciable fraction of the potassium within cells. Plasma potassium is low under these circumstances, but rarely lower than 2 mEq. per liter. In contrast the infusion of glucose causes the transfer into cells of some 30 mEq. of potassium, which though it increases the total intracellular stores insignificantly, causes a sharp fall in plasma concentration.

All diuretics, some more than others, promote the excretion of potassium. If dietary intake is adequate, little concern need be felt

about the possibility of potassium depletion. However, it should be a matter of concern if intake is inadequate and/or diuretic therapy is strenuous and prolonged. Although it is possible to measure total body stores of potassium by isotope dilution, in clinical practice it is rarely feasible. One must usually resort to a therapeutic test of the response to potassium administration in those instances in which potassium depletion is suspected.

*Chloride.* The total body chloride of a normal adult male averages 33 mEq. per Kg. of body weight. A 70 Kg. man, therefore, contains in all 2310 mEq. or about 82 gms. of chloride. The major part of this chloride (about 70 per cent) is distributed in the plasma and interstitial fluid. It can therefore be considered as being primarily an extracellular ion, but not, as has so frequently been claimed, an exclusively extracellular one. The 30 per cent of the chloride beyond the confines of the extracellular compartment is in part intracellular and in part localized in connective tissue. Of all cells, the erythrocyte contains the most chloride. Cells of the testis, ovary, gastric mucosa and skin contain lesser amounts. That present in skin may well be localized mainly in the connective tissue of the dermis. In fact collagenous fibers, wherever located, seem to be relatively rich in chloride. This has led to the description of a separate extracellular phase termed the connective tissue compartment. A reasonable view is that chloride ions are adsorbed on collagen fibers much as sodium ions are adsorbed on the apatite crystals of bone. This chloride is on the surface of the fibers, and is exchangeable.

*Bicarbonate.* The bicarbonate ion is unique among the electrolytes of the body fluids. It has no permanence as an ion species. Its existence is fleeting and is merely a step in the transfer of metabolic carbon dioxide from tissues to lungs. In fact one can best look upon bicarbonate as an anion which represents the excess of cations such as sodium, potassium, calcium and magnesium, over fixed anions such as chloride, phosphate, sulfate and protein. Since carbon dioxide and water are ubiquitous within the body and since cationic charges must be exactly neutralized by anionic charges, any cation excess (imposed by the liberation of alkali within the body) is immediately balanced by the hydration of carbon dioxide to form

bicarbonate ions. Conversely any anion excess (imposed by the liberation of acid within the body) is immediately balanced by the dehydration of bicarbonate to form carbon dioxide and water.

The total body content of bicarbonate averages 10 to 12 mEq. per Kg. About half of the total is distributed in the extracellular compartment, the remainder in tissues. The $CO_2$ in bone is largely in the form of carbonate, bound in the lattice, occluded, and largely non-exchangeable. Presumably all bicarbonate of cells is exchangeable. It is obvious that the concentration of bicarbonate in cells is only a fraction of that in extracellular fluid, for about one third of the total body store is distributed through two thirds of total body water. However, cell concentrations vary in different tissues and are known with no certainty.

## MEASUREMENT OF BODY STORES OF IONS

The total ionic content of the human body has been measured in only a few instances by complete dissolution of the cadaver and analysis of aliquots of the resulting solution. A method more appropriate to the study of normal subjects and of the alterations produced by disease is the isotope dilution method for measuring the exchangeable ion content of the body. The exchangeable ion content is less than the total ion content by the quantities of ions occluded in the crystal lattice of the bone and in minor degree, separated from plasma by the relatively impermeable blood-brain barrier.

The method for determining the exchangeable ion content of the body may be illustrated for sodium. Radioactive sodium-22 or 24 distributes throughout the body exactly as does non-radioactive sodium, except for the two reservoirs of occluded sodium noted above. If one injects some 20 to 40 microcuries of radiosodium intravenously, allows 24 hours for it to equilibrate with sodium stores of the body, withdraws a blood sample and analyzes the plasma for both radiosodium (in terms of counts per min. per ml.) and non-radiosodium (in terms of mEq. per ml.), it is possible to calculate the exchangeable sodium content of the body according to the following equation:

$$\text{Exchangeable Na (m.Eq)} = \frac{\text{counts/min. given} - \text{counts/min. excreted}}{\dfrac{\text{counts/min./ml. plasma}}{\text{mEq. Na/ml. plasma}}}$$

All urine formed during the 24 hour equilibration period is collected and an aliquot is counted to determine the quantity of radiosodium excreted. The numerator of the equation given above is the quantity of radiosodium present in the body at the time of drawing the blood sample. The denominator is the specific activity of sodium in plasma, i.e., counts/min./mEq. of sodium.

If 45,000,000 counts per min. are given as radiosodium and if 5,000,000 counts per min. are excreted in 24 hrs., there remain in the body 40,000,000 counts per min. The plasma radioactivity at this time might be 2000 counts per min. per ml. and the sodium content might be 0.140 mEq. per ml. (140 mEq. per liter). Substituting in the equation above gives:

$$\text{Exchangeable Na (mEq.)} = \frac{45{,}000{,}000 - 5{,}000{,}000}{2000/0.140} = \frac{40{,}000{,}000}{14{,}285} = 2{,}800 \text{ mEq.}$$

If the individual weighs 70 Kg., total exchangeable sodium amounts to 40 mEq. per Kg. of body weight. This method has been utilized to determine the exchangeable sodium, potassium, chloride and bicarbonate ion contents of the body.

## SUMMARY

Sodium is the major cation and chloride and bicarbonate are the major anions of plasma and interstitial fluid. These ions represent some 90 to 95 per cent of the osmotically active components of extracellular fluid. The capillary endothelium is relatively permeable to cations and to anions other than protein. Ions and water, therefore, distribute rapidly between plasma and interstitial fluid. Total osmolal concentrations and compositions of the two phases are nearly equal, differing slightly because of the presence of nondiffusible protein anions in plasma.

Potassium and magnesium are the major cations and protein and organic phosphate complexes are the major anions of intracellular fluid. Cell membranes are effectively impermeable to both anions and cations. Impermeability to protein and organic phosphate complexes is absolute. Impermeability to sodium and potassium is

relative and dependent on the continuous operation of ion pumps which extrude sodium from the cell and concentrate potassium within the cell.

Since water diffuses rapidly across cell membranes, the osmolality of intracellular fluid is essentially the same as that of extracellular fluid. Sodium diffuses into cells but is pumped out to maintain the intracellular concentration low. Potassium diffuses out of cells but is pumped in to maintain intracellular concentration high. Cellular sodium and potassium ions therefore exchange readily with their extracellular counterparts. A part of body sodium is adsorbed on the crystal lattice of bone, buried deeply in its structure, and therefore non-exchangeable. Essentially all of body potassium is exchangeable.

## REFERENCES

1. Bergstrom, W. H. and Wallace, W. M.: Bone as a sodium and potassium reservoir. *J. Clin. Invest.*, *33*:867, 1954.
2. Edelman, I. S., Leibman, J., O'Meara, M. P., and Berkenfeld, L. W.: Interrelations between serum sodium concentration, serum osmolality and total exchangeable sodium, total exchangeable potassium and total body water *J Clin. Invest.*, *37*:1236, 1958.
3. Elkinton, J. R. and Danowski, T. S.: *The Body Fluids, Basic Physiology and Practical Therapeutics.* Baltimore, Williams & Wilkins Co., 1955.
4. Glynn, I. M.: Sodium and potassium movements in human red cells. *J. Physiol*, *134*:278, 1956.
5. James, A. H., Brooks, L., Edelman, I. S, Olney, J. M., and Moore, F. D.. Body sodium and potassium I. The simultaneous measurement of exchangeable sodium and potassium in man by isotope dilution. *Metabolism, 3*.313, 1954.
6. Warner, G. F., Sweet, N J., and Dobson, E. L.: Sodium space and body sodium content, exchangeable with sodium[24] in normal individuals and patients with ascites. *Circulation Res.*, *1*:486, 1953.
7. Welt, L. G.: *Clinical Disorders of Hydration and Acid-base Equilibrium* Boston, Little, Brown & Co., 1955.

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Sodium is the major cation and chloride and bicarbonate are the major anions of plasma and interstitial fluid. These ions represent some 90 to 95 per cent of the osmotically active components of extracellular fluid. The capillary endothelium is relatively permeable to cations and to anions other than protein. Ions and water, therefore, distribute rapidly between plasma and interstitial fluid. Total osmolal concentrations and compositions of the two phases are nearly equal, differing slightly because of the presence of nondiffusible protein anions in plasma.

Potassium and magnesium are the major cations and protein and organic phosphate complexes are the major anions of intracellular fluid. Cell membranes are effectively impermeable to both anions and cations. Impermeability to protein and organic phosphate complexes is absolute. Impermeability to sodium and potassium is

plasma is 6 to 7 per cent, whereas that in interstitial fluid is 1 per cent or less. Since the capillary endothelium is more or less impermeable to proteins, they exert a colloid osmotic (oncotic) effect equivalent to a pressure of 25 mm. Hg, 25 mm. Hg so oriented as to resist filtration from the vascular compartment. Since the capillary endothelium is permeable to crystalloidal solutes, they exert no osmotic effects on fluid distribution across the vessel wall.

A third force, the tissue turgor pressure, which amounts to 2 to 5 or more mm. Hg., can also be considered as one resisting the outward filtration of fluid. The sum of these forces is equivalent to a net outward or filtration pressure of 10 to 15 mm. Hg.

Energy is expended in driving blood through the capillaries, for length is far greater in relation to diameter than is shown in the diagram. Therefore, hydrostatic pressure drops to some 10 to 15 mm. Hg. at the venular end. The oncotic effect and the tissue turgor pressure, both of which resist filtration, are essentially the same at the two ends of the capillary. Therefore, at the venular end the sum of these forces is equivalent to a net inward or absorbing pressure of 10 to 15 mm. Hg. According to the Starling hypothesis, outward filtration of fluid at the arteriolar end and reabsorption at the venular end of the capillary causes a slow circulation of fluid through the tissue interstices as illustrated in Figure 5.

***Diffusion vs. Filtration and Reabsorption in Tissue Nutrition.*** The view that tissue nutrition is largely dependent on this circulation of interstitial fluid is one which is commonly held. It is completely erroneous. It is evident from the work of Landis, Pappenheimer and others that the outward filtration of fluid at the arteriolar end of the capillary and the inward flow at the venular end are in reality quite small. Pappenheimer has calculated that pressure differences of the order of those shown in Figure 5 would cause the filtration and reabsorption of only 0.003 ml. of fluid per min. across all of the capillaries contained in 100 gm. of tissue in the human forearm, i.e., a total volume of only 40 ml. in 24 hr. It is evident from these estimates that the transport of substances to and from tissues would be extremely slow and entirely inadequate to serve their metabolic needs, if the mechanism of transport were limited

*Chapter III*

# VASCULAR AND INTERSTITIAL FLUID EXCHANGES; EDEMA

*The Starling Hypothesis.* The factors and forces determining the distribution of fluid between vascular and interstitial compartments were first clearly outlined by Starling in 1896. The elements of his thesis are represented in highly schematic form in Figure 5. There exists within the arteriolar end of the capillary a hydrostatic pressure of 40 to 45 mm. Hg. This pressure is less than aortic pressure, due to the dissipation of energy in overcoming resistance to flow in small arteries and arterioles. It represents the residual force available to drive the blood onward through capillaries,

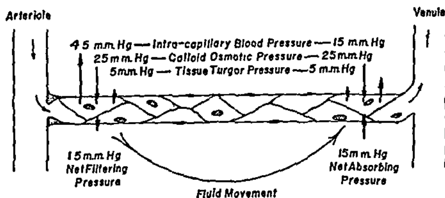



Fig. 5.

venules, and veins to the heart. It is also in part available to drive fluid outward through the porous endothelial walls of the capillaries into the interstitial spaces. However, the entire force is not available for this later purpose, for the concentration of proteins in

veins. True capillaries arise from and rejoin the preferential channels. Smooth muscle fibers at the points of origin of true capillaries serve as sphincters to control the perfusion of the capillary loops. When the sphincters relax, brisk perfusion of the loops occurs. When the sphincters contract, flow through the loops ceases.

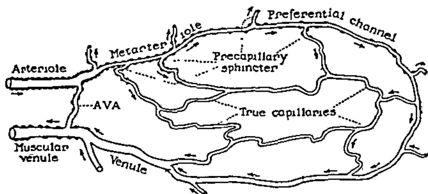



Fig. 6. Organization of the terminal vascular bed. (From B. W. Zweifach: *Tr. 3rd. Jonah Macy, Jr Conference on Factors Regulating Blood Pressure. 1949.)*

Rhythmic contraction and relaxation of precapillary spincters is observed under normal conditions and is termed vasomotion. Frequency of vasomotion and relative duration of constrictor and dilator phases together determine the minute volume of blood which perfuses a tissue and are dependent on activity of vasomotor nerves, metabolites formed in the tissues, and hormones carried in the blood stream. Zweifach has shown that during active blood perfusion, fluid filters out into the interstitium, not only at the arteriolar end but throughout the entire length of the capillary loop. During the constrictor phase, perfusion ceases, pressure drops and fluid returns to the capillary lumen throughout its length. Thus prolongation of the constrictor phase favors reabsorption of fluid, prolongation of the dilator phase favors filtration of fluid.

*Lymphatic Drainage of Interstitial Fluid.* The lymphatic system consists of a meshwork of delicate lymph capillaries ramifying through the tissue interstices. These capillaries begin in the periphery as blind endothelial tubes which progressively coalesce in their central course to form thicker walled lymphatic channels.

to bulk flow through the filtering and absorbing regions of the capillaries.

Diffusion of water and solutes in both directions across the capillary wall occurs at phenomenally high rates in comparision with the rates of transport of these substances by bulk filtration and reabsorption. Pappenheimer has calculated that the plasma contained in the capillaries of the human forearm exchanges its water with that of the surrounding interstitial fluid some 300 times per min. The sodium chloride, urea, and glucose of capillary plasma are exchanged 120, 100, and 40 times per min., respectively, with that of interstitial fluid. These rates of course describe exchange, not net transfer. However, if a tissue utilizes glucose and lowers concentration slightly in the interstitial fluid, net diffusion of glucose from plasma to tissue will occur. Similarly, any metabolite produced in the tissue will diffuse in the opposite direction when the concentration in interstitial fluid increases slightly above that in plasma.

High rates of exchange and adequate net transfer across capillary walls between blood plasma and tissues depend on the very short path over which diffusion takes place, not on extreme porosity of the endothelial membrane. According to Pappenheimer, the individual pores of muscle capillaries through which diffusion occurs have apparent diameters of 65 Å (1 Å = 1/10,000,000 mm.), and a population density of $10^9$ per $cm^2$. However, pore orfices make up only 0.1 per cent of the endothelial surface, 99.9 per cent of the surface is impermeable to water and solutes. Rapid diffusion depends on the fact that the pores are only 600 Å in length (thickness of the capillary endothelium).

*Role of Vasomotion in Filtration and Reabsorption of Interstitial Fluid.* The concept of filtration of fluid at the arteriolar end of a capillary and of reabsorption at the venular end, as illustrated in Figure 5, is no doubt a gross over-simplification. Zweifach has shown that the functional organization of the terminal vascular bed can be more adequately described in terms of the diagram shown in Figure 6. Arterioles and metarterioles lose their investments of smooth muscle, continue directly as endothelial loops termed preferential channels, and ultimately become venules and

hold the capillary open against a minute hydrostatic pressure gradient across the capillary wall. On the other hand, pinocytosis (cell drinking of fluid) and phagocytosis observed in a variety of cells in tissue culture, might account for transfer of fluid and particulate matter into the lumen of the lymph capillaries. Suffice to say that, although the forces at play are unknown, interstitial fluid enters terminal lymphatics and progresses centrally to be discharged into the superior caval venous reservoir. Centripetal flow is assisted by muscular activity in the extremities, by abdominal tone, and by the suction pump of the thoracic bellows, and is directed by a series of valves scattered along the lymphatic channels. Pulsatile lymph hearts, especially prominent in amphibia, are absent in mammals.

***Rate of Lymph Flow.*** The flow of lymph under normal conditions and especially at rest is quite small in the mammal. The flow in man, measured in individuals with fistulae of the thoracic duct, varies from 1 to 2 ml. per min. Judging from results on dogs, well over half of total lymph flow is derived from the liver and gut. It is clear that if any appreciable volume of fluid is filtered through capillary loops during the dilator phase of vasomotion, it must be rather completely reabsorbed into the same or other loops during the constrictor phase. Obviously the lymphatic system is relatively unimportant for the removal of fluid from tissues of mammals. However, continued leakage of protein and reabsorption of protein free fluid would soon build up the colloid concentration of interstitial fluid to levels which would interfere with the normal oncotic effects operating to retain fluid in the vascular system.

## FACTORS FAVORING EXPANSION OF INTERSTITIAL FLUID VOLUME

***Prolongation of the Dilator Phase of Vasomotion*** at the expense of the constrictor phase leads to the collection of excessive amounts of interstitial fluid in the tissues, for as pointed out by Zweifach, fluid filters through the walls of actively perfused capillaries along their entire length, not solely at their arteriolar ends as postulated by Starling. Hyperemia is characterized by prolongation of the dilator phase of vasomotion relative to the constrictor phase. Therefore hyperemia, whether its cause be inflam-

Channels from the lower extremities combine in the abdomen with those from the viscera to form the cisterna chyli, a multilocular plexus of vessels in the celiac region. Lymph from the cisterna chyli flows into the thoracic duct, a well defined vessel lying to the left of the midline posteriorly, to enter the venous system at the junction of the left subclavian and jugular veins. Lymph from the left head and neck, from the left upper extremity, and from thoracic structures on the left flows into the thoracic duct before it enters the venous system. A similar but less extensive system draining the right thorax, right upper extremity and right side of the head and neck drains into the right subclavian vein. Scattered along the smaller tributaries are lymph glands which subserve the several functions of mechanical removel of particulate matter, formation of lymphocytes and production of antibodies.

As has been pointed out previously, the protein concentration of lymph derived from skin and muscle is low, that from gut is intermediate, and that from liver is high. Differences in concentration are obviously an expression of differences in permeability of the capillaries of these several regions to protein. The concentration of protein is always higher in plasma than in interstitial fluid. Short of active secretion, there is no way to return protein to the vascular compartment other than by a system of drainage canals. A major function of the lymphatic system appears to be the return of protein from the interstitial to the vascular compartment.

The thin walled endothelial tubes making up the terminal ramifications of the lymphatic system are evidently much more permeable to proteins, colloidal dyes and even particulate matter than are the blood capillaries, for these materials are readily picked up and removed from the interstitial spaces by the lymphatics. The nature of the forces which operate to cause the entry of colloid-containing fluid and especially particulate matter into the lymph channels is unknown. One finds it difficult to explain such entry entirely on the basis of hydrostatic (tissue turgor) pressure, for such a pressure would be exerted equally inside and outside the lymph capillary in a fluid filled system. However, the endothelial walls of the lymph capillaries are attached to neighboring tissue cells and collagen fibers by fine protoplasmic strands. Perhaps these

*Increase in Capillary Permeability* with loss of protein into tissue interstices reduces the differential oncotic effects which ordinarily restrain fluid in the vascular compartment; i.e., the protein concentration of the interstitial fluid approaches that of the plasma. A number of factors account for the relative impermeability of normal capillaries. Protein (possibly fibrinogen, adsorbed in a mono-molecular film on the inner surface of the capillary, leak-proofs it. The endothelials cells are locked together in a tongue and groove arrangement and the chinks are filled with calcium proteinate cement impermeable to colloids. The endothelial cells themselves possess tone or turgesence which enables them to withstand the hydrostatic force distending the capillary. If tone is reduced, or conversely if compliance is increased, the capillary loses its normal impermeability to protein. Physical damage by heat or ultraviolet light, capillary poisons, increased acidity of blood, anoxia, lack of calcium, and the liberation of proteolytic enzymes and histamine in damaged tissues all increase capillary permeability to protein and thus increase transudation of fluid.

*Increased Tissue Distensibility,* i. e., loss of tissue elasticity in the aged or in those who have suffered weight loss favors expansion of extracellular fluid volume. Stretching of subcutaneous tissues during a previous episode of edema predisposes to subsequent bouts. Laxness of connective tissue around the eyes favors the development of suborbital and periorbital edema. Tissue turgor is one of the forces in the Starling hypothesis which resists outward filtration in actively perfused capillaries and which favors reabsorption in those minimally perfused.

*Obstruction of Lymphatic Drainage* of a degree sufficient to produce manifest and persistent edema is almost always the result of long standing chronic or repeated acute inflammatory processes. Lymph edema is of a brawny type, characterized by marked connective tissue proliferation and by the collection of proteinaceous fluid in the tissue interstices. It is seen in classical form in elephantiasis due to filarial infestation. In lesser degree it is seen following repeated attacks of erysipelas. Lymphatics have a very great capacity for regeneration and only repeated insults result in permanent lymphatic blockage and lymph edema.

mation, the local injection of histamine or the local application of heat, is accompanied by a local increase in formation of interstitial fluid. Three of the cardinal signs of inflammation, *rubor* (redness) and *calor* (heat), both related to increased blood flow, and *turgor* (increased interstitial fluid volume and pressure) are causally related.

*Increase in Intracapillary Hydrostatic Pressure* increases the net filtering force which causes transudation of fluid into tissues. Arteriolar dilation results in an increase in filtration pressure. Less of the potential energy imparted by the beat of the heart is dissipated in overcoming frictional resistance to flow in the arterioles; more is available to filter fluid through capillary walls. An increase in filtration pressure likewise results from an increase in venous pressure. If venous outflow is obstructed, pressure rises in the capillary bed and transudation of fluid increases. The rise in venous pressure may be local, affecting a single extremity, e.g., thrombophlebitis of the femoral vein, or it may be general and affect all capillary beds, e.g., chronic congestive heart failure. However, in congestive failure edema first collects in dependent parts of the body in which, as a result of gravity, the intracapillary hydrostatic pressure is greatest.

*Reduction in Concentration of Serum Proteins* reduces the colloid osmotic effect which operates to hold fluid within the vascular compartment. In the classical interpretation of Starling, low serum protein concentration favors excessive filtration at the arteriolar end of the capillary and reduced reabsorption at the venular end. According to Zweifach, it favors excessive filtration in actively perfused capillaries and reduced reabsorption in those which are not perfused. Low serum protein concentration is characteristic of severe protein starvation, chronic liver disease with cirrhosis and the nephrotic syndrome. In the latter condition, low serum proteins result from the massive loss of albumin in the urine. The normal 2 : 1 ratio of albumin/globulin in the serum is reduced and even reversed. Since the oncotic effect of the serum proteins is largely dependent on the albumin concentration, it suffers a larger decrement in the nephrotic syndrome than might be anticipated from the degree of reduction in total protein concentration.

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*The Edemas of Nephrosis, Cirrhosis and Nephritis* were early explained along similar lines. In the nephrotic syndrome, massive loss of albumin in the urine was thought to lead to hypoproteinemia, reduced plasma oncotic pressure, increased transudation, edema and prerenal diversion of fluid in causal sequence. The edemas of severe protein starvation and of cirrhosis were likewise considered to be due at least in part to hypoproteinemia. However, in cirrhosis the factor of increased portal pressure was recognized as of major significance. In nephritis, emphasis was placed on a vasculitis affecting not only the terminal vascular bed of the kidneys but that of other viscera and of systemic structures as well. This vasculitis, increasing capillary permeability to protein, resulted in a reduction in the oncotic effect which holds fluid within the vascular compartment. Again the significant sequence was increased transudation, edema and prerenal diversion of fluid.

## MODERN CONCEPTS OF THE PATHOGENESIS OF EDEMA

Present concepts of the pathogenesis of edema differ from those just presented more in emphasis than substance. The major differences relate to the role of the kidneys in the retention of salt and water and to the sequence of events leading to the formation of edema.

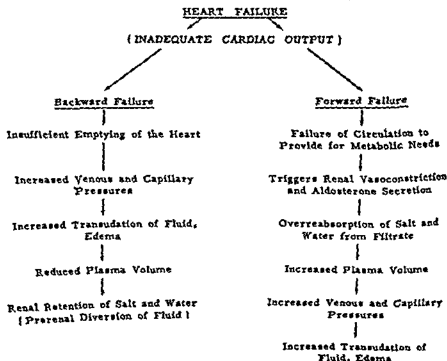
***Role of the Kidneys in the Pathogenesis of Edema.*** The concept that prerenal diversion of fluid into tissue interstices accounts for renal retention is a semantic simplification without justification of fact. In even the most severely edematous patient, the amount

## EARLY CONCEPTS OF THE PATHOGENESIS OF EDEMA

For the first four decades of this century the view was commonly held that generalized edema, whatever its cause, could be more or less completely explained in terms of Starling's hypothesis. It was maintained that one or more of the forces favoring capillary transudation, namely increased hydrostatic pressure, reduced plasma oncotic pressure or increased capillary permeability is primarily increased in edematous patients. Salt and water are retained in the body because that which is absorbed by the gut is diverted into the tissues as edema, little is presented the kidneys for excretion. A necessary corrolary of increased transudation and prerenal diversion of fluid as primary causes of edema is relative anhydremia and oligemia. By no means are these universal findings.

*Backward Failure of the Heart.* The general features of the pathogenesis of edema outlined above may be illustrated in terms of the concept of backward failure of the heart, summarized on the

TABLE III
THE PATHOGENESIS OF EDEMA IN CONGESTIVE HEART FAILURE



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## MODERN CONCEPTS OF THE PATHOGENESIS OF EDEMA

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*Role of the Kidneys in the Pathogenesis of Edema.* The concept that prerenal diversion of fluid into tissue interstices accounts for renal retention is a semantic simplification without justification of fact. In even the most severely edematous patient, the amount

of salt and water presented to the kidneys in the renal arterial blood is many orders of magnitude greater than that which would have to be excreted to achieve fluid balance. The normal individual achieves fluid balance by excreting each day a fraction of one per cent of the salt and water delivered into the renal tubules in the glomerular filtrate. More than 99 per cent of the quantities filtered is reabsorbed. Although the severely edematous patient may form less glomerular filtrate each day, balance could be achieved by the excretion of a very few per cent of the filtered quantities. Instead little salt and less than normal quantities of water are excreted during the phase of accumulation of edema. It is evident that absolute or relative over-reabsorption of salt and water from the glomerular filtrate, not prerenal diversion, ultimately accounts for renal retention.

It is now apparent that two alterations in renal function in varying degree underlie the failure of edematous patients to excrete salt and water. Certain patients have low rates of glomerular filtration. Less than normal quantities of sodium and water are delivered into the renal tubules each day, yet tubular reabsorption continues at nearly the usual rate. As a consequence, all is reaborbed; none is excreted. Other edematous patients have normal rates of glomerular filtration. Tubular reabsorption of salt and water is enhanced, in fair part due to stimulation by excessive production of adrenal salt retaining hormones; as a consequence, excretion is reduced. These two factors, reduced filtration and hormonally stimulated over-reabsorption, appear to be synergistic causes of *retention of salt and water in congestive heart failure, cirrhosis, the* nephrotic syndrome, acute nephritis, and eclampsia, and no doubt are operative in other forms of generalized edema as well. However, the relative roles of these two factors differ from patient to patient and from time to time in a given patient. The nature of these alterations in renal function will be considered in greater detail in Chapter VI.

If renal retention of salt and water is of primary significance, one would expect to observe plethora and hydremia in edematous patients; both are common findings. A part of the retained fluid filters out into the tissues in accordance with the balance of forces

outlined in Starling's hypothesis. It is distributed first to sites of highest hydrostatic pressure and lowest tissue turgor pressure. Rate of accumulation is enhanced by hypoproteinemia and by increased capillary permeability. While his successors placed major emphasis on the balance of forces across capillary walls in explaining the pathogenesis of edema, as long ago as 1896 Starling suggested the possibility that diminished excretion of fluid by the kidneys could result in hydremia and that hydremia might be the cause of increased effective capillary filtration pressure and edema.

*Forward Failure of the Heart.* The general features of the pathogenesis of edema outlined above may be illustrated in terms of the concept of forward failure of the heart, summarized on the right of Table III. As a consequence either of intrinsic disease of the heart, which reduces its work capacity, or of extrinsic demands, which exceed its work tolerance, the cardiac output becomes inadequate to satisfy the metabolic demands of the body. Thus failure may develop in the presence of reduced cardiac output (valvular heart disease, acute or chronic myocarditis; arteriosclerotic heart disease) or of increased cardiac output (thyrotoxicosis, beriberi, severe anemia, massive arterio-venous fistula). One must postulate that failure of the circulation to provide for metabolic needs triggers mechanisms for salt and water conservation. These mechanisms include renal vasoconstriction, which reduces renal blood flow and glomerular filtration rate, and stimulation of secretion of adrenal steroids, which promote renal tubular reabsorption of salt. Salt and water are retained in the vascular compartment inducing hydremia and plethora. Venous and effective capillary filtration pressures rise. As a consequence of increased transudation, interstitial fluid volume increases.

The weak element of the argument is that as yet no mechanism has been satisfactorily described which senses adequacy of cardiac output relative to metabolic demands and which translates that information into the neural and hormonal control of tubular reabsorption of salt and water. However, the fact that such a mechanism has not been characterized does not deny its existence. In favor of the hypothesis are the following facts. Starr has shown in patients dying in congestive failure that the adynamic filling pres-

of salt and water presented to the kidneys in the renal arterial blood is many orders of magnitude greater than that which would have to be excreted to achieve fluid balance. The normal individual achieves fluid balance by excreting each day a fraction of one per cent of the salt and water delivered into the renal tubules in the glomerular filtrate. More than 99 per cent of the quantities filtered is reabsorbed. Although the severely edematous patient may form less glomerular filtrate each day, balance could be achieved by the excretion of a very few per cent of the filtered quantities. Instead little salt and less than normal quantities of water are excreted during the phase of accumulation of edema. It is evident that absolute or relative over-reabsorption of salt and water from the glomerular filtrate, not prerenal diversion, ultimately accounts for renal retention.

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prolongation of the dilator phase of vasomotion, by an increase in capillary permeability, by a reduction in serum colloids, by increased tissue distensibility and by obstruction of lymphatic drainage. Ultimately the extent of edema formation is determined by the avidity with which the kidneys retain ingested salt and water.

## REFERENCES

1. Laragh, J. H., Gilman, A., and Cross, R. J.: Mechanisms of edema formation and principles of management. *Am. J. Med., 21:*423, 1956.
2. Pappenheimer, J. R.: Passage of molecules through capillary walls *Physiol. Rev., 33:*387, 1953.
3. Peters, J. P.: The problem of cardiac edema. *Am. J. Med., 12:*66, 1952.
4. Starling, E. H.· *The Fluids of the Body*. Herter Lectures, Chicago, W. T. Keener & Co., 1909.
5. Zweifach, B. W.: Basic mechanisms in peripheral vascular homeostasis. *Tr 3rd Josiah Macy, Jr. Conf. on Factors Regulating Blood Pressure,* New York, Macy, 1949, p. 13.

sure of the circulatory system is increased, i.e., the mean pressure in the vascular bed after the heart has stopped beating is elevated. This implies that the circulatory system is overfilled during life. Most studies on living patients utilizing dilution methods described earlier, have shown that circulating blood volume is increased. Furthermore, in patients developing edema, renal retention of salt and water frequently precedes elevation of venous pressure. Primacy of renal retention of fluid, stimulated by relative inadequacy of cardiac output, has the virtue of explaining edema in those conditions in which high output failure exists, e.g., thyrotoxicosis, beriberi, severe anemias and massive arteriovenous fistulae, as well as in those with low output failure.

## SUMMARY

Fluid is distributed between the vascular system and the interstitial compartment in accordance with the balance of forces across the capillary endothelium. The hydrostatic pressure of the blood within the capillaries is the force responsible for the outward filtration of fluid. This is balanced by the colloid osmotic effect of the plasma proteins and by the tissue turgor pressure which tend to hold fluid in the peripheral vascular bed. According to the classical theory of Starling, fluid leaves the blood stream at the arteriolar end of the capillaries, where high hydrostatic pressure favors outward filtration, and reenters at the venular end, where low hydrostatic pressure favors absorption. Zweifach has modified this concept by showing that fluid filters outward along the entire length of the capillary during active perfusion of tissues with blood and is absorbed along the entire length during the quiescent period. Alternate perfusion and quiescence of circulation is brought about by vasomotion of the terminal vascular bed. Relatively little fluid is returned to the vascular compartment by the lymphatics. However, the lymphatics play an important role in that they permit the return to the blood of the protein which leaks through the capillary walls.

Transudation of fluid and formation of edema is favored by an increase in capillary hydrostatic pressure due either to arteriolar dilation or to the obstruction of venous return. It is favored by

prolongation of the dilator phase of vasomotion, by an increase in capillary permeability, by a reduction in serum colloids, by increased tissue distensibility and by obstruction of lymphatic drainage. Ultimately the extent of edema formation is determined by the avidity with which the kidneys retain ingested salt and water.

## REFERENCES

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4. Starling, E. H.: *The Fluids of the Body.* Herter Lectures, Chicago, W. T. Keener & Co., 1909.
5. Zweifach, B. W.: Basic mechanisms in peripheral vascular homeostasis. *Tr. 3rd Josiah Macy, Jr. Conf. on Factors Regulating Blood Pressure*, New York, Macy, 1949, p. 13.

*Chapter IV*

# MECHANISMS OF RENAL FILTRATION, REABSORPTION, AND EXCRETION OF IONS AND WATER

THE kidneys regulate the volume, osmolality, and ionic composition of the extracellular fluid. If water or any of the major extracellular ions, sodium, chloride, and bicarbonate is present in the body in excess, it is eliminated in the urine; if the body store is deficient, excretion is curtailed. Urine formation begins with the ultrafiltration of large volumes of plasma through the glomerular capillary tufts. To prevent rapid exhaustion of body stores, the bulk of the filtered ions and water must be reabsorbed from the tubular urine. When intake is within the usual range, only a small fraction, less than one per cent, of the filtered sodium, chloride, bicarbonate, and water need be excreted to achieve balance. If less than normal quantities are reabsorbed, body reserves are progressively depleted and dehydration results. If reabsorption is excessive, body reserves progressively expand and edema develops. As a prelude to a description of the abnormalities of volume regulation and of renal function in edema, we shall consider in this chapter the normal renal processes of glomerular filtration, tubular reabsorption, and excretion of ions and water.

## GLOMERULAR ULTRAFILTRATION OF IONS AND WATER

Simple filtration removes particulate matter from plasma; glomerular ultrafiltration carries the process a step further and removes colloids (proteins and lipids). However, an ultrafiltrate like a simple filtrate contains all crystalloidal solutes in essentially the same concentrations as exist in the aqueous phase of the original fluid. True, since the filtrand (plasma) contains protein anions while the ultrafiltrate contains none, the concentrations of crystalloidal anions (chloride and bicarbonate) will be slightly higher in

the filtrate than in the aqueous phase of plasma. Conversely the concentrations of crystalloidal cations (sodium and potassium) will be slightly lower in the filtrate than in the aqueous phase of plasma.[10] These differences in concentration, which are relatively small, are manifestations of the Gibbs-Donnan equilibrium described earlier on page 14. Uncharged crystalloids like glucose and urea have identical concentrations in the ultrafiltrate and in the aqueous phase of plasma.

*Criteria of Ultrafiltration* are three: (1) the ultrafiltrate must be protein free; (2) it must contain all crystalloids in exactly the same concentrations as in the aqueous phase of the plasma, except for the slight deviations demanded by the Gibbs-Donnan rule; and (3) the hydrostatic force must be adequate to account for the volume of ultrafiltrate formed per unit time. The general acceptance of glomerular ultrafiltration as the initial process in urine formation is based on the admirably direct and precise studies of Dr. A. N. Richards and his colleagues on the amphibian and mammalian kidney. Richards devised the method of puncturing Bowman's capsule of a glomerulus with a micro glass pipette, sealing off the neck of the tubule with a glass rod to prevent reflux, and collecting the filtrate by gentle aspiration as it forms.

Wearn and Richards first noted that glomerular filtrate collected in this manner from the amphibian kidney is protein-free, an observation extended to the mammalian kidney[11] by Walker, Bott,

[10]As a rough approximation, the concentrations of chloride and bicarbonate in an ultrafiltrate of normal plasma are calculated as 1.05/0 94 times the concentrations in plasma; the concentrations of sodium and potassium in an ultrafiltrate are calculated as 0 95/0.94 times the concentrations in plasma. The divisor, 0 94, corrects each expression for the water content of plasma, i e., the ions in 1 ml. of plasma are dissolved in 0 94 ml. of water.
The dividends, 0 95 and 1 05, are the Donnan factors for univalent cations and anions, respectively, applied when ion concentrations are expressed in mEq. per unit volume of water Both the Donnan factors and the water correction are based on a plasma protein concentration of 6 per cent and a normal plasma pH.

[11]Actually the filtrate contains no protein by the test method employed which had a lower limit of sensitivity of 30 mg per cent. The protein concentration of mammalian plasma is some 6000 mg. per cent. Since the filtrate contains less than 0 5 per cent of the protein content of plasma, it is reasonable to say that it is protein free. Actually trace amounts of protein probably are filtered and absorbed by the renal tubules. Certain mammals, including the rat, normally filter and excrete significant amounts of protein.

Oliver and MacDowell. This satisfies the initial criterion of glomerular ultrafiltration, i.e., the filtrate must be protein-free.

Richards and his colleagues then devised a series of microchemical methods applicable to the microliter or so of fluid which could be collected from a single glomerulus. They clearly demonstrated that the concentrations of glucose, chloride, sodium, urea, phosphate, uric acid and creatinine are the same in the filtrate as in the aqueous phase of the plasma. They likewise observed identity of pH, electrical conductivity and osmolal concentration. These observations satisfy the second criterion of glomerular ultrafiltration, namely that the concentrations of crystalloids are the same in plasma and filtrate.

The third criterion, i.e., adequacy of hydrostatic force, although satisfied by experimental measurements in the amphibian kidney, *must at present be accepted more on probability than rigorous* proof in the mammalian kidney. In man, with a mean arterial pressure of 100 mm. Hg. mean glomerular capillary pressure has been estimated to be 75 mm. Hg. This high value derives from the *short and direct arterial supply to the glomeruli*, the afferent arterioles arising directly from the interlobular arteries. The colloid osmotic effect of plasma proteins, equivalent to a pressure of 25 to 30 mm. Hg, and the intrarenal turgor pressure of 15 mm. Hg oppose the intracapillary hydrostatic pressure. The balance of forces yields a net filtration pressure of 35 to 40 mm. Hg. This pressure is available to overcome frictional resistance in forcing fluid through the minute pores of the glomerular capillary membrane and along the tubule from glomerulus to pelvis. According to calculations of Pappenheimer, the forces are adequate. If each glomerulus produced only one drop of filtrate in a day's time, the total filtrate formed in the 2.5 million glomeruli of two normal human kidneys would add up to the 150 to 200 liters now known to be formed in 24 hours.

***Properties of the Glomerular Capillary Filter.*** Pappenheimer has developed an operational concept of the structure of the glomerular capillary membranes, based on his studies of their relative permeability to water and to a series of substances of varying molecular dimensions. They behave as though they were per-

forated by myriads of aqueous channels, cylindrical in form, and some 75 Å in diameter by 600 Å in length. Glomerular capillaries are far more permeable to water and solutes than are muscle capillaries. Pappenheimer ascribes this to the fact that the aqueous pores occupy 5 per cent of the surface of glomerular capillaries, only 0.1 per cent of the surface of muscle capillaries. The glomerular pores are also slightly larger. The total glomerular capillary surface of man has been estimated to be 8,000 to 16,000 $cm.^2$; the total pore area is therefore 400 to 800 $cm.^2$.

The diameter of the pores is such that they restrain absolutely serum globulin, but not free hemoglobin nor albumin. Yet little hemoglobin and much less albumin (only traces) enter the filtrate. Pappenheimer explains the sieving of these molecules, of a diameter approaching that of the pores, on the basis of probability. It is unlikely that a hemoglobin molecule, with an effective diameter[12] of 64 Å, will hit a pore of 75 Å sufficiently "head on" to enter. A few do. The likelihood that albumin, with an effective diameter of 70 Å, will do so is even less. Very few do. A second factor restricting passage of molecules into the filtrate is viscous drag as they traverse aqueous channels. The greater the diameter of the molecule relative to that of the channel, the greater the drag.

Water and the common crystalloids of plasma have very small molecular and ionic diameters relative to pore dimensions, of the order of only a few Ångstrom units. Therefore the crystalloids are unrestricted in their entry into pores and in their passage along the pores relative to water. Accordingly, the capillary membrane removes colloids from the filtrate, yet permits free passage of crystalloids and water. At least two ions, calcium and magnesium are in part bound to plasma protein. That fraction which is bound to protein (40 to 60 per cent) is restrained from entering the filtrate; the free fraction dissolved in the aqueous phase traverses the membrane without restriction except for a minor Donnan effect.

[12]The effective diameter is the Einstein-Stokes diameter, i.e., the diameter of a sphere which has *equivalent diffusion* characteristics. Neither hemoglobin nor albumin is spherical, yet their diffusion properties are the same as spheres of 64 Å and 70 Å diameter.

Oliver and MacDowell. This satisfies the initial criterion of glomerular ultrafiltration, i.e., the filtrate must be protein-free.

Richards and his colleagues then devised a series of microchemical methods applicable to the microliter or so of fluid which could be collected from a single glomerulus. They clearly demonstrated that the concentrations of glucose, chloride, sodium, urea, phosphate, uric acid and creatinine are the same in the filtrate as in the aqueous phase of the plasma. They likewise observed identity of pH, electrical conductivity and osmolal concentration. These observations satisfy the second criterion of glomerular ultrafiltration, namely that the concentrations of crystalloids are the same in plasma and filtrate.

The third criterion, i.e., adequacy of hydrostatic force, although satisfied by experimental measurements in the amphibian kidney, must at present be accepted more on probability than rigorous proof in the mammalian kidney. In man, with a mean arterial pressure of 100 mm. Hg. mean glomerular capillary pressure has been estimated to be 75 mm. Hg. This high value derives from the short and direct arterial supply to the glomeruli, the afferent arterioles arising directly from the interlobular arteries. The colloid osmotic effect of plasma proteins, equivalent to a pressure of 25 to 30 mm. Hg, and the intrarenal turgor pressure of 15 mm. Hg oppose the intracapillary hydrostatic pressure. The balance of *forces yields a net filtration pressure of 35 to 40 mm. Hg.* This pressure is available to overcome frictional resistance in forcing fluid through the minute pores of the glomerular capillary membrane and along the tubule from glomerulus to pelvis. According to calculations of Pappenheimer, the forces are adequate. If each glomerulus produced only one drop of filtrate in a day's time, the total filtrate formed in the 2.5 million glomeruli of two normal human kidneys would add up to the 150 to 200 liters now known to be formed in 24 hours.

*Properties of the Glomerular Capillary Filter.* Pappenheimer has developed an operational concept of the structure of the glomerular capillary membranes, based on his studies of their relative permeability to water and to a series of substances of varying molecular dimensions. They behave as though they were per-

irregular oval apertures from 400 to 900 Å in diameter. The pores, about 1/100 the diameter of a red cell, are of course far too large to account for impermeability of the membrane to colloids. Because of the multiple perforations, the endothelial layer is termed the lamina fenestra.

The middle or basement membrane layer consists of a uniformly dense, apparently structureless, osmophilic tube (the dark-staining layer 3), sandwiched between two concentric tubes of osmophobic material (unstained layers 2 and 4.) Although Hall has described pores in the basement membrane with diameters of the order of 100 Å, others have not observed them, and it is probable that those seen by him were artifacts. The three-layered basement membrane, also called the lamina densa, is some 600 Å thick. It seems likely that this membrane accounts for the colloid impermeability of glomerular capillaries, even though stable pores of the proper dimensions are not demonstrable.

The concept of a pore as a cylindrical channel of fixed dimensions is an operational one. There is evidence that water itself is structured; i.e., the molecules are arranged in a lattice held together by hydrogen bonds. Substances diffuse through water by jumping from position to position in the lattice. It is possible that the basement membrane is a protein-water-phospholipid lattice and that water and solutes traverse it by jumping from position to position, not by bulk flow through continuous cylindrical tubes. If so, it is not surprising that such a tenuous structure cannot be demonstrated by electronmicrography.

The outer layer of the glomerular capillary wall is made up of podocytes (ep. in Fig. 7) and their interdigitating cytoplasmic extensions, the trabeculae and pedicels (layer 1). These podocytes can be considered as "octopus-like" cells sitting on the tube of basement membrane and grasping it with interlocking arms, the trabeculae. The trabeculae carry numerous pedicels which are intimately applied to the lamina densa. They fit closely together leaving the basement membrane exposed through narrow slits. Rhodin believes that these narrow slits between pedicels permit passage of water and crystalloids but restrain colloids. According to this view, protein impermeability would be determined by the

*The Electron Microscopic Structure of Glomerular Capillaries* unfortunately does not give any clear cut confirmation of the operational picture outlined above. Pores 75 Å in diameter and 600 Å in length should be readily identifiable in electron micrographs were they geometrically stable elements of the capillary wall and were staining methods adequate to distinguish wall substance and pore. It is possible that one or both of these conditions is not met.

According to Hall, Pease, Yamada, and Rhodin, the glomerular capillary is made up of three layers, illustrated diagrammatically in Figure 7. The inner layer consists of the soma and cytoplasmic extensions of endothelial cells. The soma of the endothelial cell,

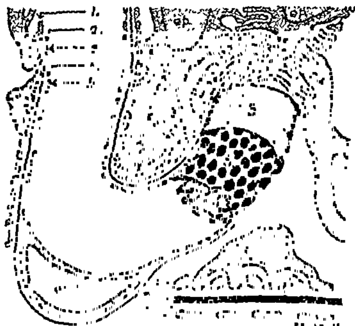



Fig. 7. Schematic representation of the structure of a glomerular capillary as revealed by the electron microscope. (From D. C Pease. *J. Histochem & Cytochem.* 3:259, 1955.)

containing a large oblate nucleus, lies eccentrically and bulges into the lumen of the capillary. Away from the nucleus, the cytoplasm spreads out into a film some 250 to 500 Å in thickness, forming a continuous endothelial tube (layer 5). This tube is perforated by

irregular oval apertures from 400 to 900 Å in diameter. The pores, about 1/100 the diameter of a red cell, are of course far too large to account for impermeability of the membrane to colloids. Because of the multiple perforations, the endothelial layer is termed the lamina fenestra.

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outermost podocyte layer rather than by the basement membrane. Unfortunately no decision can be reached at the moment as to whether the lamina densa or the podocyte processes account for selective permeability of the capillary wall. To the author it would appear more reasonable to consider the epithelial cells and their trabeculae as reenforcements, necessitated in glomerular capillaries by high intravascular pressure.

*The Rate of Glomerular Filtration* averages 125 ± 25 ml. per min. per 1.73 $M^2$ surface area in the adult male and 110 ± 15 ml. per min, per 1.73 $M^2$ in the adult female. Filtration rate varies as some function of body size but whether specifically as a function of surface area has never been firmly established. It decreases with advancing age in the absence of cardiovascular, renal or hepatic disease.

Rate of glomerular filtration is a highly significant datum for an understanding of the pathophysiology of renal disease, for the quantification of renal tubular reabsorption and secretion, and for the assessment of the functional defects which underlie the formation of edema. The clearance of inulin is the accepted measure of the rate of glomerular filtration in man and in all other forms studied to date. In the dog, the creatinine clearance is equal to and has the same functional significance as the inulin clearance and is technically a simpler measurement to perform. Unfortunately in man, the creatinine clearance is not identical under all circumstances with the inulin clearance, for a small amount of creatinine is secreted by the renal tubules. Furthermore a part of the apparent endogenous creatinine of plasma is not creatinine and is reabsorbed by the renal tubules.

The concept of renal plasma clearance was developed by Van Slyke and his associates in the course of their investigation of the mechanism of excretion of urea. They defined the urea clearance as the volume of plasma completely cleared of urea by the kidneys in one minute's time. Rehberg first pointed out that the renal plasma clearance of a substance would equal the rate of glomerular filtration if that substance exhibited certain well defined properties. Although he correctly described the requisite properties, the substance he chose, creatinine, failed to exhibit them in man. Some

years later Shannon and Smith and Walker and Richards simultaneously proposed inulin as fulfilling the necessary requirements, a prediction which has been amply confirmed in subsequent investigations.

*The Properties of a Substance Whose Clearance Is to Measure Rate of Glomerular Filtration* are the following: (1) It must be freely filterable through the pores of the glomerular capillary membrane; i.e., it must be a crystalloid, not bound to plasma proteins and of such dimensions that it is not sieved appreciably in passing through the capillary wall. Inulin with an Einstein-Stokes diameter of 30 Å is about the largest molecule which could freely pass pores 75 Å in diameter without significant sieving. In fact inulin may be sieved to a slight but quantitatively insignificant degree. (2) The substance must be sufficiently large and insoluble in the lipid phase of the tubular epithelium so that it will not be passively reabsorbed. Due to reabsorption of water, inulin becomes highly concentrated in the terminal portions of the renal tubules. Despite high concentration gradients between tubular lumen and peritubular fluid, there is good evidence that inulin does not diffuse back into the bloodstream. (3) The substance must be inert; the renal tubules must neither actively reabsorb nor secrete it. A variety of lines of evidence indicates that inulin meets these requirements. (4) It must be non-toxic, i.e., it must not in itself alter renal function. Providing it is pyrogen-free, inulin meets this requisite. (5) Finally it must be determinable in plasma and urine with a high degree of accuracy. Analytical methods for inulin are satisfactory when carefully applied.

*The Principles Involved in the Measurement of Glomerular Filtration Rate* are illustrated diagrammatically in Figure 8. Since the kidney consists of many nephrons in parallel, certain functions can be illustrated most simply in terms of a single nephron, many times the size and functional capacity of the actual unit. If 125 ml. of plasma are filtered by this composite glomerulus each minute and if each ml. of plasma contains 1 mg. of inulin, it is obvious that 125 mg. of inulin will be delivered into the renal tubule each minute. Suppose that the urine flow is 1 ml. per min., and that no inulin is reabsorbed and none secreted as the filtrate flows along

the renal tubule. Each minute 125 mg. of inulin will be excreted in 1 ml. of urine. The clearance is defined as the rate of excretion (urine concentration × urine flow) divided by the plasma concentration:

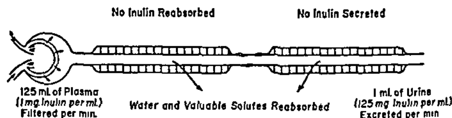



*Fig. 8. Principles involved in the measurement of rate of glomerular filtration by the inulin clearance method.*

$$C_{in} = \frac{U_{in} \times V}{P_{in}}; \text{ where } C_{in} =$$

*clearance of inulin in ml. per min;* $U_{in}$ = *urine inulin concentration* in mg. per ml.; V = urine flow in ml. per min.; and $P_{in}$ = *plasma concentration of inulin in mg. per ml. Substituting* the values given above:

$$C_{in} = \frac{125 \text{ mg./ml.} \times 1 \text{ ml./min.}}{1 \text{ mg./ml.}} = 125 \text{ ml. per min.}$$

*It is evident that the inulin clearance so calculated is the same as* the rate of glomerular filtration assumed in the example cited. *Several factors must be rigorously controlled if measured inulin* clearances are to approximate rates of glomerular filtration satis-*factorily:* plasma inulin concentration must be held constant over the period of measurement; urine collections must be complete and accurately timed; no sudden changes in urine flow should occur during the course of clearance measurement; blood samples should be frequent and accurately timed; the analysis of inulin in plasma and urine must be accurate.

It is necessary to point out that equating plasma inulin clearance with glomerular filtration rate is to some extent ambiguous. The inulin clearance is a plasma clearance, yet the aqueous phase, not whole plasma, is filtered through the glomeruli.. If one wishes to

ured inulin clearance ($C_{in}$) must be multiplied by the fraction of water in plasma (normally 0.94 at a plasma protein concentration of 6 per cent).

*The Quantities of Ions Filtered per Minute* can be readily calculated if one measures simultaneously glomerular filtration rate ($C_{in}$) and the plasma concentrations of the several ion species ($P_{Na}$, $P_K$, $P_{Cl}$ and $P_{HCO_3}$). The products of these two variables times the appropriate Donnan factor divided by 1,000 (since plasma concentrations of ions are expressed in mEq. per liter) yield the quantities filtered in mEq. per min.[11] The quantities filtered per min. multiplied by 1440 yield the quantities filtered per 24 hrs. Table IV summarizes average values for the quantities of potassium and of the major extracellular ions filtered per minute and per day. In each instance the quantities filtered per day far exceed the quantities present in the extracellular compartment. With the exception of potassium, the quantities filtered also considerably exceed total body stores.

## TUBULAR REABSORPTION OF IONS AND WATER

*Magnitude of the Reabsorptive Problem.* The filtration of ions and water in the copious amounts shown in Table IV demands nearly equivalent tubular reabsorption to prevent rapid exhaustion of body stores. The magnitude of the reabsorptive problem is summarized in the columns on the right of this table. If an individual is in water and electrolyte balance, he must excrete each day in the urine the quantities of ions and water ingested, minus whatever quantities are eliminated by extrarenal routes. The remainder of that filtered must be reabsorbed by the renal tubules. If an individual ingests 2500 ml. of water in food and drink and loses 1000 ml. by extrarenal routes, it is obvious that he must

[11] In calculating quantities of ions filtered, one does not correct for the fraction of plasma which is water. The reason is that ion concentrations are expressed per unit volume of plasma and glomerular filtration rate is expressed as ml of plasma filtered per min. The product of $C_{in}$ (ml. plasma per min) and $P_{Na}/1000$ etc. (mEq. per ml. of plasma) times the Donnan factor gives the quantity of ions filtered directly. However, if one wishes to calculate the concentrations of ions in the filtrate, it is necessary to correct for the difference in water content of plasma and filtrate and for Donnan distribution as well.

TABLE IV

Quantities of Ions and Water Filtered, Excreted, and Reabsorbed by the Kidneys of Man (Mean Normal Values)

| | *Plasma Conc.* | *Donnan Factor* | *Glom Filt. Rate* | *Quantity Filtered* | | *Quantity Excreted* | *Quantity Reabsorbed* | *Per Cent Filtered Reabsorbed* |
|---|---|---|---|---|---|---|---|---|
| | *(mEq./L.)* | | *(ml /min)* | *(mEq./min)* | *(mEq./24 hr)* | | | |
| Sodium | 140 | 0 95 | 125 | 16.6 | 23,900 | 171 | 23,729 | 99.3 |
| Chloride | 103 | 1.05 | 125 | 13.5 | 19,500 | 171 | 19,329 | 99.1 |
| Bicarbonate | 27 | 1 05 | 125 | 3.55 | 5,100 | 2 | 5,098 | 99.9 |
| Potassium | 4 | 0.95 | 125 | 0 475 | 684 | 51 | 633 | 80.6 |
| | *Plasma Fraction* | | | *(ml /min.)* | *(ml /24 hr.)* | | | |
| Water | 0 94 | | 125 | 118 | 169,000 | 1,500 | 167,500 | 99.1 |

reabsorb 167,500 ml. of the 169,000 ml. filtered, excreting only 1500 ml. Over 99 per cent of the filtered water is reabsorbed. A luxus intake of sodium chloride is 10 gm. or 171 mMols per day; that of potassium is 2 gm. or 51 mEq. In the absence of sweating or diarrhea, extrarenal losses are minor and may be neglected. The reabsorption of filtered sodium and chloride ions is more than 99 per cent complete, that of potassium, 80 per cent complete. The net dietary intake of bicarbonate is less than zero for the usual diet is acid ash, i.e., it contains more potential acid anions than cations. One and one half liters of urine of pH 5.5 to 6.0 contains about 2 mEq. of bicarbonate. Thus over 99.9 per cent of the filtered bicarbonate is reabsorbed; essentially none is excreted.

## ION AND WATER TRANSPORT MECHANISMS IN RENAL TUBULAR CELLS

*General Features of Ion and Water Reabsorption.* The mechanisms of reabsorption of ions by the renal tubules are no doubt related to, and modifications of mechanisms present in the membrane of every cell. In Chapter II, the characteristic differences in composition of extracellular and intracellular fluids were ascribed to the operation of membrane pumps which extrude sodium from and concentrate potassium within the cell. In most cells, sodium enters by diffusion and is actively extruded through all free surfaces. In renal tubular cells, sodium enters mainly from the tubular lumen and is extruded into the peritubular fluid. The tubular transport system is oriented to pump sodium unidirectionally.

If sodium is pumped from lumen to peritubular fluid, a potential difference will be established across the tubular epithelium which will favor diffusion of anions in the same direction, i.e. downhill along an electrical gradient. Therefore, it is unnecessary to postulate independent active transport mechanisms to account for the reabsorption of the major anions, chloride and bicarbonate. While this thesis will be developed below, it must be emphasized that it has not as yet been established by any rigorous proof.

The active pumping of sodium from lumen to peritubular fluid coupled with the passive diffusion of anions will establish an osmotic force across the tubular epithelium which will favor the

diffusion of water in the same direction. If the tubule is relatively permeable to water, ions and water will be transported at equivalent rates, and although volume of tubular urine will suffer progressive reduction, osmolality will remain unchanged. If, on the other hand, the tubule is impermeable to water, the reabsorption of ions will reduce the osmolality of the tubular fluid causing it to become hypotonic, whereas the immediate peritubular fluid into which ions are pumped will become hypertonic. There is now reason to believe that sodium is actively transported from lumen to peritubular fluid in the proximal segment, in the thin segment of the loop of Henle, in the distal segment and in the collecting duct as well. The proximal tubule is permeable to water under all conditions; therefore, the residual tubular urine remains isosmotic and reabsorption of sodium results in a reduction in volume. The thin segment of the loop of Henle, at least its ascending segment, is impermeable to water; therefore, the residual tubular urine becomes hypotonic, whereas the immediate peritubular fluid becomes hypertonic. The distal tubule and the collecting duct are variably permeable to water. Under conditions of water diuresis, i.e., in the absence of circulating antidiuretic hormone, the distal tubule and collecting duct are impermeable to water; a large volume of hypotonic urine is excreted. Under conditions of hydropenia, i.e., in the presence of a high titre of circulating antidiuretic hormone, the distal tubule and collecting duct are permeable to water; a small volume of hypertonic urine is excreted.

For some time the view has been held that hypertonic urine is formed by the active reabsorption of solute-free water from a small volume of isotonic fluid delivered from the distal segment into the collecting duct. Two theoretical difficulties argue against an active water pump. one, the conceptual difficulty of visualizing a carrier mechanism which will transport water; the other, the extremely rapid turnover required of any carrier complex concerned with water transport. The transport of 1.0 ml. of isotonic saline per min. by a mechanism which actively pumps sodium and which permits osmotic equilibration of water would require roughly $10^{20}$ successive combinations and dissociations of sodium with carrier. Transport by a mechanism which actively pumps

water and which permits the passive diffusion of sodium would require more than 300 times this number of successive combinations and dissociations of water and carrier. This results from the fact that isotonic saline is 55.5 Molar with respect to water, but only 0.150 Molar with respect to sodium.

Recently Wirz, Hargitay and Kuhn have explained the formation of hypertonic urine in terms of the active transport of sodium from the ascending limb of the loop of Henle into the peritubular interstitial fluid of medulla and papilla, rendering it hypertonic. Water equilibrates across the epithelium of the collecting ducts which traverse this hypertonic tissue; the final urine becomes equally hypertonic. This thesis, revolutionary to say the least, was not generally accepted at first. However, confirmation of its basic tenets by Ullrich, Berliner, Gottschalk and others makes its consideration imperative. We shall develop it in more detail below.

*The Basic Characteristics of Ion and Water Transport in the Proximal Tubule* have been defined in micropuncture studies begun by Dr. A. N. Richards and his colleagues nearly 30 years ago and continued in recent years by Drs. Bott, Wirz, Gottschalk and others. Clearance studies under conditions of osmotic loading, of water loading, and of altered acid base metabolism have provided much ancillary information.

First, reabsorption in the proximal segment is isosmotic; i.e., solutes and water are reabsorbed at equivalent rates. This statement is based on the original observations of Walker, Hudson, Findley and Richards on the amphibian nephron and those of Walker, Bott, Oliver and MacDowell on the mammalian nephron. It has subsequently been confirmed by Wirz and by Gottschalk. All groups have observed that the glomerular filtrate is isosmotic with plasma and remains so as if flows along the proximal tubule, even though volume is reduced to a fraction of its original value.

Second, ion reabsorption is primary and active; water reabsorption is secondary and passive; i.e. water transport depends largely on osmotic forces set up by the reabsorption of ions. Four lines of evidence support this statement. (1) Wesson and Anslow in experiments on dogs have shown that the rapid infusion of a hypertonic solution of mannitol may increase urine flow to 30 to 40 ml. per

min., i.e., to a value equal to half of filtration rate. At a time when 50 per cent of the water entering the renal tubules in the glomerular filtrate is excreted in the urine, only 8 per cent of the filtered bicarbonate, 22 per cent of the filtered sodium, and 33 per cent of the filtered chloride are excreted. The urine is isotonic[14] and, as claimed by Wesson and Anslow, may represent proximal tubular fluid relatively unmodified[15] during its rapid transit through the distal segment. If this assumption is correct, then the net reabsorption of ions in the proximal segment exceeds the net reabsorption of water and at least one and perhaps all of these ions are actively reabsorbed. (2) Solomon and Giebisch have measured potential differences of some 20 to 40 millivolts between tubular lumen (negative) and peritubular fluid (positive) in the Necturus and rat. Such potentials are most readily explained in terms of active transport of sodium ions, a thesis which will be developed in detail below. (3) Windhager *et al.* have shown by "stopped flow" perfusion of proximal tubules of Necturus with isotonic mixtures of mannitol and saline in varying proportions that sodium and chloride ions can be actively reabsorbed to establish a gradient of 2:3 between lumen and peritubular fluid. (4) In preliminary micropuncture studies on the rat under conditions of mannitol diuresis, Giebisch and Windhager have found that sodium and chloride ions are reabsorbed against significant concentration gradients, as great as 1·2. Points (3) and (4) above constitute proof of active transport of ions across the proximal tubule. Since the tubular fluid remains isotonic, i.e. since the concentration of mannitol is increased in proportion to the reduction in concentration of ions, it is reasonable to conclude that water is reabsorbed osmotically and secondary to the reabsorption of ions.

Third, reabsorption is essentially isohydric under normal conditions; the pH of the glomerular filtrate changes little as it flows along the proximal segment. This fact has been directly demonstrated by Montgomery and Pierce only for the amphibian neph-

[14]The osmotic deficit created by the reabsorption of ions was exactly balanced by the increase in mannitol concentration which resulted from water reabsorption.
[15]The low bicarbonate content of the urine may in part be due to rapid reabsorption in the distal tubule. Hence "unmodified proximal tubular fluid" can be applied to the final urine only with significant reservations.

ron. In the absence of evidence to the contrary, it has been inferred for the mammalian nephron. Results of Ullrich et al and of Pitts et al are consonant with this view, for they indicate that the site of major acidification in the mammalian as well as in the amphibian nephron is distal. If proximal reabsorption is isohydric, bicarbonate and water must be reabsorbed at equivalent rates; i.e. no significant change in bicarbonate concentration occurs in the proximal segment during reabsorption of the bulk of the filtrate. No doubt the proximal epithelium permits the free diffusion of carbon dioxide. If bicarbonate concentration and pCO2 remain unchanged, pH is also unchanged.

It has recently been shown by Gottschalk that in marked osmotic diuresis in the rat, produced by the infusion of 25 per cent glucose, proximal tubular fluid may become 0.6 to 0.8 pH units more acid than blood plasma, an observation confirmed by Giebisch and Windhager in mannitol diuresis as well. However, these latter investigators have shown that in diuresis induced by the infusion of isotonic saline, no significant acidification of proximal fluid occurs. As has been noted above, a sodium gradient as great as 1:2 is established between tubular fluid and peritubular blood plasma in profound osmotic diuresis. One would predict an equivalent anion gradient, and Giebisch and Windhager have observed one for chloride roughly equal to that for sodium. If a comparable bicarbonate gradient were also established, the pH of proximal urine would be 0.3 to 0.4 units below that of plasma. In contrast, in saline diuresis no significant sodium or chloride gradient develops across the proximal tubule. One would, therefore, predict no bicarbonate nor pH gradient and none of appreciable magnitude has been found.

The condition of saline diuresis more closely approximates the normal than does osmotic diuresis, for little or no sodium gradient is established under normal conditions or in saline diuresis, whereas an appreciable gradient develops in osmotic diuresis. However, it should be pointed out that the degree of proximal acidification in osmotic diuresis is roughly twice that explainable on the basis of a bicarbonate gradient equal to that for sodium. Accordingly, bicarbonate must be preferentially reabsorbed with respect to

either sodium or chloride in osmotic diuresis. Furthermore, Giebisch and Windhager have shown that in saline diuresis combined with respiratory acidosis, proximal tubular urine is acidified to a degree equivalent to that observed in osmotic diuresis. Therefore under two conditions, osmotic diuresis and respiratory acidosis, bicarbonate is preferentially reabsorbed and the tubular urine acidified. In saline diuresis under conditions of normal acid base balance and probably in non-diuretic normal conditions as well, no preferential reabsorption of bicarbonate and no acidification of the urine occurs. The significance of these findings with respect to the mechanism of bicarbonate reabsorption is not clear at the moment.

Fourth, under normal conditions, i.e., in the absence of osmotic diuresis, no significant concentration gradients are established between proximal tubular fluid and plasma for any of the three ions, sodium, chloride and bicarbonate. This view is based on micropuncture studies on both amphibian and mammalian nephrons. Most would agree that the sodium concentrations of proximal tubular fluid and of plasma are essentially equal. Recent work of Giebisch and Windhager has demonstrated that the chloride concentrations are the same. The observations, described in the paragraphs above, suggest that the bicarbonate concentrations are also the same. However, it must be reiterated that identity of concentration of ions in proximal fluid and plasma holds only under normal conditions and in saline diuresis, not in osmotic diuresis, and not in respiratory acidosis.

Fifth, there occurs a marked reduction in volume of the tubular contents as fluid flows along the proximal segment of the mammalian nephron. According to Walker, Bott, Oliver and MacDowell, *some 4/5ths and perhaps, as Smith claims, as much as* 7/8ths of the ions and water are reabsorbed.

The characteristics of proximal tubular reabsorption of ions and water outlined above are implicit in the diagram of Figure 9. Thus reabsorption of sodium is active, that of water is passive and dependent on osmotic forces set up by the reabsorption of sodium. The glomerular filtrate is isotonic with plasma and the tubular fluid remains isotonic as it flows along the convoluted and straight

portions of the proximal segment. Volume at the end of the proximal tubule is reduced to a quantitatively uncertain degree, but probably to a value within the range of 1/5th to 1/8th that of the glomerular filtrate. If we assume that 125 ml. of plasma are filtered through the glomeruli each minute, 118 ml. of plasma water per minute would be delivered into the proximal segments of the renal tubules. Sodium ions along with chloride and bicarbonate ions in equivalent quantities are reabsorbed. Glucose, phosphate, uric acid, amino acids, vitamins and other normal constituents of the blood plasma are more or less completely reabsorbed. Water is osmotically reabsorbed and volume is reduced to perhaps 15 to 20 ml. per min. at the end of the proximal tubule.

It is well to reemphasize here that the osmolality of the plasma and glomerular filtrate is in large part due to its content of sodium, chloride and bicarbonate ions. Therefore, it is the reabsorption of these ions which creates the major fraction of the osmotic force that causes water reabsorption. A minor fraction is contributed by the reabsorption of glucose, amino acids, phosphate and sulfate. Excretory products such as urea, creatinine, uric acid, etc. are concentrated to some degree by the reabsorption of fluid in the *proximal segment. Theoretically they would be concentrated some* 5 to 8 times, practically somewhat less, for a fraction of the filtered urea (according to Shannon, some 20 to 40 per cent) diffuses back into the blood across the proximal tubular epithelium. Obviously, these excretory products exert a greater proportion of the osmotic pressure of the tubular fluid at the end of the proximal segment than they do at its beginning. Accordingly sodium, chloride and bicarbonate ions must exert a somewhat smaller proportion of total osmotic pressure; i.e., their concentrations must be slightly lower at the end than at the beginning of the proximal tubule.

*An Alternative View of Passive Reabsorption of Proximal Fluid.* Bayliss and more recently Malvin et al have suggested that proximal reabsorption of fluid (water and sodium and chloride ions) is passive and due to the colloid osmotic force exerted by the plasma proteins in the peritubular capillaries and the crystalloid osmotic force developed in consequence of the active reabsorption of glucose, amino acids, and other so-called threshold solutes. This

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Fifth, there occurs a marked reduction in volume of the tubular contents as fluid flows along the proximal segment of the mammalian nephron. According to Walker, Bott, Oliver and MacDowell, some 4/5ths and perhaps, as Smith claims, as much as 7/8ths of the ions and water are reabsorbed.

The characteristics of proximal tubular reabsorption of ions and water outlined above are implicit in the diagram of Figure 9. Thus reabsorption of sodium is active, that of water is passive and dependent on osmotic forces set up by the reabsorption of sodium. The glomerular filtrate is isotonic with plasma and the tubular fluid remains isotonic as it flows along the convoluted and straight

when the urinary load of excretory solutes is within the usual normal range. However, in profound osmotic diuresis induced by the infusion of mannitol, the concentration of osmotically active excretory products in the glomerular filtrate may be increased from a normal value of 6 or 7 mOsm. per liter to 60 or more mOsm. per liter. If the only force available to reabsorb fluid in the proximal tubule were that represented by the 7.0 mOsm. per liter of peritubular colloids and actively reabsorbed threshold solutes, 10 per cent or less of the filtrate could be returned to the blood stream. The reabsorption of 10 per cent of the fluid would concentrate the mannitol by 10 per cent and the process would stop. No gradient of concentration of sodium and chloride ions could be established by such a mechanism.

As mentioned earlier, Windhager *et al.* and Giebisch *et al.* have observed that a gradient of concentration of sodium and chloride ions develops along the proximal tubule of both the Necturus and rat when mannitol is present in the tubular fluid. Such a gradient permits the reabsorption of considerably more fluid than the 10 per cent noted above. Furthermore, the development of a gradient demands active transport of one or both ions.

The basic question is not whether the above mentioned oncotic and osmotic forces, equivalent to 7.0 mOsm. per liter, contribute to the reabsorption of fluid, obviously they must, but whether the tubular epithelium is so freely permeable to ions that they are unrestricted in their movements relative to water, hence exert no osmotic effects. The author believes that evidence available to date favors the view that active transport of sodium is necessary to effect the reabsorption of the bulk of the filtrate in the short time that the fluid is in contact with proximal tubular epithelium. The extent to which passive diffusion of ions and water occurs down the slight oncotic and osmotic gradient contributed by plasma proteins and by reabsorbed threshold substances is undetermined. The bidirectional fluxes of sodium, potassium, and chloride across the tubule of the frog and Necturus observed by Hoshiko et al, Chinard *et al.*, Whittenbury *et al.*, and Giebisch and Windhager suggest that the tubule may be sufficiently permeable to these ions to permit some passive reabsorption of proximal fluid. That such

implies that the permeability of the proximal epithelium to sodium and chloride ions is of the same order of magnitude as the permeability to water, and is roughly comparable to that of the glomerular capillary membrane. This is equivalent to saying that only the excretory products in the tubular fluid exert an osmotic effect;

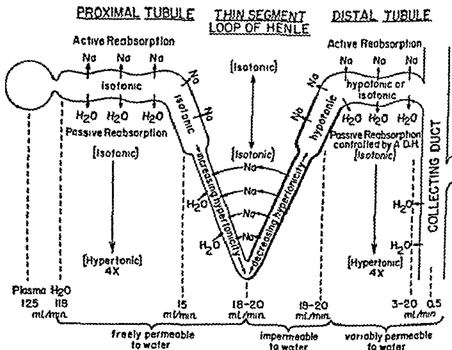



Fig 9. The functional organization of the nephron in relation to reabsorption of sodium and water and formation of dilute and concentrated urine The diagram incorporates views of Wirz, Hargitay, and Kuhn, Gottschalk, and Berliner

the tubule is freely permeable to or actively reabsorbs all other solutes. Were this true, fluid could be reabsorbed passively until the excretory products are concentrated some 7 mOsm. per liter above their concentration in the glomerular filtrate. This derives from the fact that the colloid osmotic force of the plasma proteins in the peritubular capillaries is equivalent to 2.0 mOsm. per liter and the crystalloid osmotic force of actively reabsorbed threshold solutes is equivalent to 5.0 mOsm. per liter. Were all assumptions valid, such a passive mechanism could theoretically account for the reabsorption of half to two-thirds of the glomerular filtrate

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passive reabsorption is a significent fraction of the total is doubtful.

*Ion Transport in the Thin Segment of the loop of Henle.* A series of rather surprising observations, at first difficult to accept, but now amply confirmed, has necessitated a revision of concepts of the function of the thin segment of the loop of Henle. A bit of historical review may help to give perspective.

Marshall and his colleagues a number of years ago pointed out the following two facts: only birds and mammals have a true loop of Henle interposed between proximal and distal convoluted tubules; only birds and mammals can form urine hypertonic to blood plasma. They postulated that the urine is concentrated in the thin segment of the loop of Henle by the active reabsorption of water, and that water transport is specifically stimulated by antidiuretic hormone. If this view were correct, urine collected by micropuncture from any portion of the nephron distal to the thin segment should be as hypertonic to plasma as is the final urine.

Subsequently, it was observed that the urine collected from the distal convoluted tubules may be isotonic or even hypotonic when the ureteral urine is hypertonic; concentration must therefore be the final step in the elaboration of urine and must take place in the collecting ducts. Smith postulated that the thin segment of the loop of Henle plays only a passive role, ensuring osmotic equilibration of proximal tubular urine with blood plasma prior to its delivery into the distal segment. According to Smith the urine is concentrated in the collecting ducts by the active reabsorption of water from a small volume of isotonic fluid.

Certain rodents, especially the desert rat, can form urine which is far more hypertonic to the plasma than is that of dog and man. These forms have a long urinary papilla, made up of thin segments of loops of Henle interspersed with collecting ducts and capillary loops, which projects into a greatly elongated renal pelvis. Evidence has recently accumulated that the medullary tissue and especially the urinary papilla are very significantly hypertonic to the renal cortex and to other tissues. Thus Glimstedt and Ljungberg noted that slices of medullary tissue taken in succession from the corticomedullary junction to the tip of the urinary papilla

contain increasing amounts of chloride. Wirz, Hargitay and Kuhn observed that the osmotic pressure of such slices progressively increases to reach a maximum at the tip of the papilla. Ullrich noted that urea is highly concentrated in the papilla and Levinsky made similar observations with respect to sodium. Finally Wirz noted that blood collected from surface capillaries of the papilla is hypertonic to general systemic blood. The hypertonicity of medullary and papillary tissue cannot be ascribed solely to hypertonic urine contained in the collecting ducts. The tissue itself must be hypertonic. The thesis of Wirz and more recently that of Gottschalk is that sodium pumps located in the thin segments of the loops of Henle extrude sodium ions (plus equivalent numbers of chloride ions) into the interstitium of the medulla and urinary papilla rendering it hypertonic to plasma. Water is abstracted osmotically from the urine in the collecting ducts until its osmotic pressure increases to equal that of the surrounding papillary interstitial fluid.

*Counter-Current Multiplication of Concentration in the Loops of Henle.* The details of loop function, shown in the center section of Figure 9, follow in general the concept of Wirz, Hargitay and Kuhn and of Gottschalk. The concept is that of a counter current concentration multiplier. Details of operation are illustrated in Figure 10. Isotonic urine is delivered into the descending limb of the loop of Henle from the proximal tubule. From the corresponding level of the ascending limb, sodium is actively extruded into the interstitium, reducing concentration in the ascending limb, increasing concentration in the interstitium. According to Wirz, sodium may be actively secreted into the descending limb; according to Gottschalk it merely diffuses into the descending limb down a small gradient of concentration. The epithelium of the ascending limb from the tip of the loop well into the cortex must be impermeable to water, otherwise water would follow sodium osmotically and no change in concentration would result. This feature is indicated in Figures 9 and 10, by the heavy line forming the wall of the ascending limb. The descending limb, in contrast, is probably permeable to water.

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The significant event as one follows the two limbs of Henle's loop from the cortico-medullary junction to the tip of the papilla is the transfer of sodium from ascending limb to descending limb, active extrusion from the ascending limb and either active secretion or passive diffusion into the descending limb. The significant

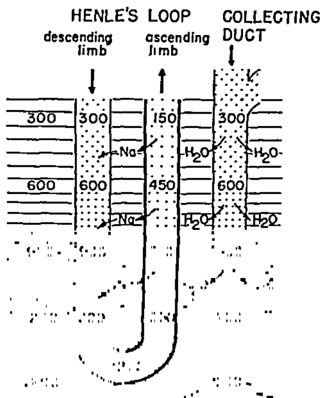



Fig. 10. The role of counter current multiplication of concentration in the loop of Henle in the elaboration of hypertonic urine.

consequences of cyclic recirculation of sodium are: progressively increasing osmolar concentration as the urine flows down the descending limb; progressively decreasing concentration as urine flows up the ascending limb; and progressively increasing osmolality of the interstitial fluid from the cortico-medullary junction to the tip of the papilla. An especially intriguing feature of this hypothesis is that at no point within the system must sodium be

pumped against a high concentration gradient. The maximum osmotic gradient (4 to 1 for the human kidney) is developed longitudinally over the entire length of Henle's loop. The diagrams of Figures 9 and 10 give an erroneous impression of the volume of interstitial fluid in the papilla. In histological section, volume is minimal; tubules and capillaries are densely packed. Therefore, minimal amounts of sodium need be sequestered in the interstitial fluid of medulla and papilla to render it hypertonic.

As was pointed out above, hypertonicity of medullary and papillary tissue has been demonstrated by several investigators. Wirz has shown that blood collected from papillary capillaries is hypertonic. Gottschalk has recently demonstrated by micropuncture of loops of Henle near the papillary tip that the tubular urine is equally hypertonic. The basic elements of the thesis of Wirz and Gottschalk have, therefore, been established directly. At present there is no valid means of determining whether transfer of sodium into the descending limb is active (Wirz) or passive (Gottschalk). The system proposed by Wirz would operate more effectively in that the sodium pumps of the ascending and descending limbs of Henle's loops would operate in series, the system proposed by Gottschalk does not involve active reabsorption in one limb of Henle's loop and active secretion in the other, an assumption which is philosophically somewhat hard to accept.

***Counter-current Exchange in Capillary Loops of the Papilla.*** One might reasonably predict that the flow of blood in capillaries supplying the medulla and papilla would so rapidly dissipate the hyperosmolality of tissue and interstitial fluid that it would be ineffective as a means of concentrating the urine during final transit through the collecting ducts. Wirz first suggested that the arrangement of capillaries in the medulla and papilla is such as to permit their functioning as counter-current exchangers of diffusible solutes. Counter-current exchange considerably reduces the effect of blood flow in dissipating the osmotic gradient. Furthermore, it is probable that only a minor fraction of total renal blood flow perfuses the medulla and papilla. Low total flow and counter-current exchange are both important for the maintenance of hyperosmolality of medullary and papillary tissue.

Berliner has illustrated the operation of the counter-current vascular loop in terms of a thermal model presented in Figure 11, A. B. Consider the tube shown in diagram A on the left, through which water flows at a constant rate of 10 ml. per min. A source supplies heat at a rate of 100 calories per min. If the fluid entering the system has a temperature of 30°C., that leaving the system will have a temperature of 40° C. If the tube is bent upon itself, as shown in diagram B, insulated to prevent heat loss to the outside, but so arranged as to permit free transfer of heat between emergent and entering streams, certain features of operation will

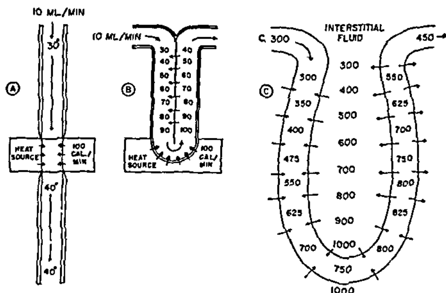



Fig. 11. The principal of counter current exchange. A. Thermal model without counter current exchange. B. Thermal model incorporating counter current exchange. C. *Counter current exchange in medullary capillary loops as a means of preservation of tissue hypertonicity.* (From R.W. Berliner, N.G. Levinsky, D G. Davidson, and M Eden· *Am. J. Med.*, 24 730, 1958.)

change. If rate of fluid flow, the addition of heat, and the temperature of the entering stream are the same, the temperature of the emergent stream will also be the same. However, the temperature at the heat source will be much higher in the counter-current system, for some of the heat will be transferred from the outflowing to the inflowing streams. If one considers the function of the stream of water to be that of cooling the heat source, it is

evident that the straight through system on the left is more effective than the counter-current system. In other words, so far as its cooling effect on the heat source is concerned, the counter-current arrangement has reduced the effective flow to a small fraction of the actual flow.

The capillary shown in diagram C on the right illustrates the operation of the counter-current loop in terms of its effect in preserving the osmotic gradient in the renal medulla. Blood enters the loop with an osmotic concentration of 300 mOsmols per liter. As the capillary dips into the medullary and papillary interstitium with its high osmolal concentration, osmotically active particles diffuse into the blood. As blood traverses the loop and *ascends, osmotically active particles diffuse out into the interstitium.* The loop operates to reduce the effective blood flow with respect to dissipation of the interstitial osmotic gradient. Furthermore, total blood flow in the papilla is probably low. These two factors are significant in permitting the development of, and for the maintenance of an osmotic gradient.

*Ion and Water Transport in the Distal Tubule.* Walker, Bott, Oliver and MacDowell first showed by micropuncture of the nephron of the rat that the fluid delivered into the first part of the distal tubule is hypotonic to plasma even when the final urine is hypertonic. This fact was later confirmed by Wirz and by Gottschalk (*cf.* Fig. 9). If we accept the hypothesis of Wirz that sodium is actively transported into the descending limb of Henle's loop, then most of the water reabsorbed from the collecting ducts into the papillary interstitium will follow the sodium osmotically. Volume flow in the ascending limb of the loop and in the first part of the distal segment may be estimated to be 18 to 20 ml. per min. It will be greater than the volume entering the loop of Henle from the proximal tubule by the amount of water reabsorbed from the collecting ducts. On the other hand, if we accept the hypothesis of Gottschalk that sodium diffuses passively into the descending limb of Henle's loop, then the water reabsorbed from the collecting ducts will largely enter the blood capillaries of the medulla and papilla; the volume leaving the loop of Henle may not differ greatly from the volume entering it. The point of

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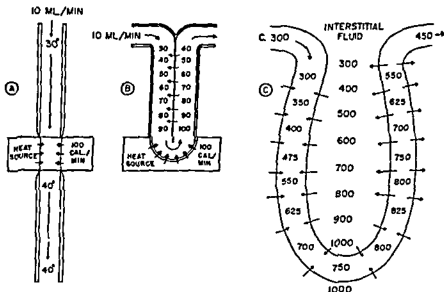



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As it flows along the collecting duct the tubular fluid gives up water to the medullary and papillary interstitium. Volume is reduced to 0.3 to 0.5 ml. per min. and osmolar concentration is increased to 1200 to 1400 mOsmols per liter, essentially that of the papillary tissue.

## NATURE OF MECHANISMS FOR TUBULAR TRANSPORT OF IONS

In all of the preceding discussion it is implied if not explicitly stated that the tubular transport of sodium is primary and active, that of chloride and bicarbonate secondary and passive. We may loosely define active transport of an ion as net movement of that ion from a region of lower to a region of higher electrochemical potential; passive transport, as net movement from a region of higher to a region of lower electrochemical potential. While this definition by no means covers all possibilities, it is adequate for our purposes. That sodium transport by the renal tubules is active, i.e., uphill against an electrochemical gradient, is highly probable. That chloride and bicarbonate transport is downhill along an electrical gradient will become apparent when we consider potential differences between tubular lumen and bloodstream. That there is no active transport of these anions has not been firmly established. However, the assumption of active sodium reabsorption and passive chloride and bicarbonate reabsorption is reasonable, for it brings the basic transport mechanisms of renal tubular cells into line with those of red cells, muscle cells and nerve cells. Smith, Weston, Berliner and others have suggested this possibility. We shall develop the thesis in somewhat more detail, borrowing liberally from the transport mechanism of frog skin outlined by Ussing and from those of red cells, muscle cells and nerve cells described by Shaw, Glynn, Hodgkin, Keynes and others.

Ussing has pointed out that certain purely physical forces apart from active transport can operate to cause the net transfer of ions across membranes. These include, (1) differences in concentration, (2) differences in activity coefficients, (3) differences in electrical potential between phases in contact with the membrane and

difference is an academic one and it makes little difference which view is accepted. The degree of hypotonicity of the fluid entering the first part of the distal tubule will be conditioned by the rate of uptake of solute free water from the collecting ducts and by the rate of loss of salt from the medullary interstitium to blood flowing in the papillary capillary loops. Counter-current exchange of solute in capillary loops is of course not completely effective and any salt lost to the blood must be replaced from the fluid in the ascending limb of Henle's loop. Since the tubular fluid remains hypotonic to a point where the convolutions of the distal segment approach the parent glomerulus, it is evident that the epithelium to this point must be impermeable to water.

As shown in Figure 9, sodium reabsorption continues in the distal tubule. In the maximally hydrated individual, the concentration of circulating antidiuretic hormone is minimal and the distal tubule and collecting ducts are impermeable to water. Continued reabsorption of sodium reduces concentration to a very low value and a large volume of dilute urine is excreted. The rate of urine flow will be roughly the same as the rate at which proximal tubular fluid is delivered into the descending limb of *Henle's loop. Relatively little fluid will be gained in the loop of* Henle, relatively little lost in the distal tubule and collecting duct.

In the hydropenic individual, the concentration of circulating antidiuretic hormone is high and both distal tubules and collecting ducts are permeable to water. Hypotonic urine delivered into the distal segment loses water to the cortical interstitium. Continued reabsorption of sodium creates an osmotic force which causes further reabsorption of water. Volume is reduced from 18 to 20 ml. per min. at the beginning of the distal tubule to 3 to 6 ml. per min. at the end. The tubular fluid is isotonic as it enters the collecting ducts and contains most of the products destined for excretion.[14]

[14]Ullrich and his associates have recently shown by a remarkably ingenious method [illegible] is reabsorbed [illegible] ammonia and [illegible] of the orifices of collecting ducts at the tip of the papilla with filamentous polyethylene tubes Minute samples of urine were collected at various levels as the catheters were advanced.

the continued inward diffusion of sodium from the tubular lumen. One view would be to consider that the sodium transport mechanism is an electrogenic ion pump; i.e. the pumping of sodium directly establishes the potential difference, positive outside, negative inside. If this were true, potassium would migrate into the

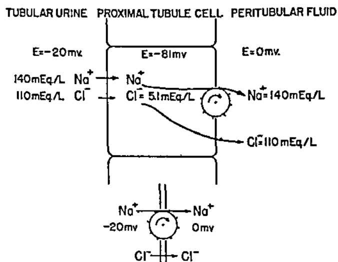



Fig. 12. Hypothetical mechanism of transport of sodium and chloride ions by proximal tubular cells. (From R.F. Pitts *Am. J. Med.*, 24.745, 1958.)

cell along this electrical gradient until the high ratio of intracellular to extracellular potassium concentration would just balance the potential difference. Another view, more in line with current thinking on frog skin, nerve, and muscle and illustrated in Figure 3 A, B would be that the sodium pump is electrically neutral due to coupling of inward movement of potassium with extrusion of sodium. The transcellular potential then becomes a potassium diffusion potential. No net flux of potassium need occur if passive outflux were to equal active influx; i.e., if the cell were to leak potassium back into the peritubular fluid as rapidly as it transports sodium.

Let us further assume that chloride is free to diffuse out of the cell into the peritubular fluid. If diffusion is free, one can calculate from the Nernst equation and the potential difference of 81 milli-

finally, (4) solvent drag force, arising from passage of solvent through the membrane. None seems capable of contributing to an understanding of the overall mechanism of active sodium transport. At least one, namely differences in electrical potential between phases in contact with the membrane, probably plays an important role in the reabsorption of chloride and bicarbonate. Solvent drag may play a role in the reabsorption of all components of the filtrate not reabsorbed solely by carrier mechanisms.

Wilbrandt, Sidney Solomon and more recently Giebisch, utilizing microelectrodes, have recorded potentials between the tubular lumen and peritubular fluid, the so-called transtubular potentials. In addition Giebisch has recorded potentials between the interior of proximal tubular cells and the peritubular fluid, the so-called transcellular potentials. Both transtubular and transcellular potentials play key roles in the thesis we wish to develop. The only complete measurements of both potential values are those of Giebisch on the amphibian kidney; however, we shall make the extrapolation, partially justified by Solomons measurements, that equal or higher potential differences occur in the mammalian kidney. In any event the reader should realize that the hypothesis we shall advance is tentative and represents merely a personal synthesis of evidence too fragmentary and incomplete to constitute proof.

*Nature of Proximal Tubular Transport of Sodium and Chloride.* According to Giebisch there exists a transcellular potential difference of 60 to 90 millivolts between the interior of proximal tubular cells and peritubular fluid in the kidney of Necturus. The interior of the cell is negative to its surroundings. A transtubular potential difference of around 20 millivolts exists between tubular lumen and peritubular fluid. The lumen of the tubule is negative to the peritubular fluid. The relationships of these potentials are represented diagrammatically in Figure 12.

Let us assume that the transcellular potential is basically established by a pump which actively extrudes sodium from the cell into the peritubular fluid. The pump operates continuously and at such a rate that the sodium content of the cell is maintained at a low value, perhaps 1/10th or less that of extracellular fluid, despite

the continued inward diffusion of sodium from the tubular lumen. One view would be to consider that the sodium transport mechanism is an electrogenic ion pump; i.e. the pumping of sodium directly establishes the potential difference, positive outside, negative inside. If this were true, potassium would migrate into the

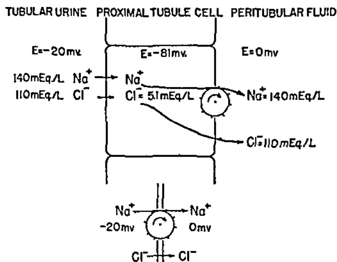



Fig. 12. Hypothetical mechanism of transport of sodium and chloride ions by proximal tubular cells. (From R.F. Pitts: *Am. J Med.*, 24 745, 1958)

cell along this electrical gradient until the high ratio of intracellular to extracellular potassium concentration would just balance the potential difference. Another view, more in line with current thinking on frog skin, nerve, and muscle and illustrated in Figure 3 A, B would be that the sodium pump is electrically neutral due to coupling of inward movement of potassium with extrusion of sodium. The transcellular potential then becomes a potassium diffusion potential. No net flux of potassium need occur if passive outflux were to equal active influx; i.e., if the cell were to leak potassium back into the peritubular fluid as rapidly as it transports sodium.

Let us further assume that chloride is free to diffuse out of the cell into the peritubular fluid. If diffusion is free, one can calculate from the Nernst equation and the potential difference of 81 milli-

finally, (4) solvent drag force, arising from passage of solvent through the membrane. None seems capable of contributing to an understanding of the overall mechanism of active sodium transport. At least one, namely differences in electrical potential between phases in contact with the membrane, probably plays an important role in the reabsorption of chloride and bicarbonate. Solvent drag may play a role in the reabsorption of all components of the filtrate not reabsorbed solely by carrier mechanisms.

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diffuse from cell to tubular lumen are buffered by bicarbonate ions to form carbonic acid. The carbonic acid dehydrates to $CO_2$ and water, the $CO_2$ diffusing into the cell to re-enter the bicarbonate cycle. Since sodium and water are reabsorbed and bicarbo-

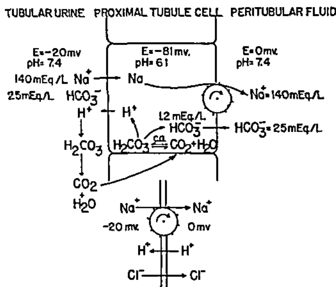



Fig 13. Hypothetical mechanism of transport of sodium and bicarbonate ions by proximal tubular cells. (From R.F. Pitts *Am. J. Med.*, 24 745, 1958.)

nate disappears at equivalent rates, the bicarbonate concentration and pH of the residual fluid will remain unchanged.

The simplified diagram at the bottom of Figure 13 illustrates the overall operation of the proximal ion reabsorptive system. Sodium is actively transported from lumen to peritubular fluid. Chloride diffuses passively in the same direction, but since its diffusion is restricted relative to sodium transport, the transtubular potential difference of 20 millivolts develops. Hydrogen ions diffuse into the tubular lumen along this electrical gradient.

Many readers may legitimately enquire why such a complicated mechanism need be postulated to explain reabsorption of bicarbonate if the rather simple one of movement down an electrical gradient suffices for chloride. The answer is that a number of

volts that the intracellular chloride concentration will be low, roughly 5 mEq. per liter. If one further assumes that there is some restriction to the diffusion of chloride into the cell from the tubular lumen relative to sodium, one can account for the transtubular potential of minus 20 millivolts. This is clearer in the simplified diagram at the bottom of Figure 12, in which the tubular epithelium is represented as a single membrane. Active transport of sodium accompanied by passive diffusion of chloride will lead to the development of a transtubular potential of proper sign, if chloride diffusion lags behind sodium transport. Were chloride to diffuse as fast, it would short circuit the sodium pump and no transtubular potential would exist. We feel that this transtubular potential plays an important role in the sodium-hydrogen exchange process which Berliner, Schwartz, Gilman, and Pitts believe to be important in the reabsorption of bicarbonate in the proximal tubule. One obvious ambiguity in Figure 12 is that we have cited amphibian potentials and mammalian ion concentrations. This is done purposely to emphasize the fact that we are illustrating a concept, not attempting to prove a thesis.

*Nature of Proximal Tubular Transport of Bicarbonate.* The cell represented in Figure 13 might well be the same one shown in Figure 12. However in this second diagram, sodium and bicarbonate reabsorption rather than sodium and chloride reabsorption is illustrated. The sodium pump establishes the transcellular potential of 81 millivolts. Let us assume that the luminal border of the cell is essentially impermeable to bicarbonate ion but that the peritubular border is as permeable to bicarbonate as to chloride. We can then calculate that the internal bicarbonate concentration will be low, of the order of magniture of 1.0 mEq. per liter. This is equivalent to the Boyle and Conway formulation of the Donnan distribution of bicarbonate in muscle. If the cell is permeable to dissolved $CO_2$, the pH of its contents will be roughly 6.1. Hydrogen ions are therefore more than 20 times as concenrated within the cell as in the tubular urine. They will diffuse outward from the cell into the tubular lumen despite the net electrical gradient of 61 millivolts opposing diffusion. No active transport of hydrogen ions would necessarily be required. The hydrogen ions which

ever, the observation of modest acidification of proximal tubular urine does not demand active transport of hydrogen ions.

***Nature of Distal Tubular Transport of Sodium, Hydrogen and Potassium.*** The distal transport mechanisms are undoubtedly much more complicated than the proximal mechanisms just described. This statement derives from the following facts. First, in forming urine of pH 4.4 from plasma of pH 7.4, a process now definitely localized to distal portions of the nephron in both the amphibian and mammalian kidney, hydrogen ions must be transported against a gradient of 1,000 to 1. Were this a passive transfer, it would demand a transtubular potential of the order of 180 millivolts, a value far in excess of any measured by Solomon or Giebisch in either the rat or Necturus. Second, in forming dilute urine, distal tubular cells can remove sodium almost completely from the tubular fluid, certainly against a gradient of 50 to 1. Third, as Berliner, and others have shown, hydrogen ions and potassium ions compete for the sodium exchange mechanism.

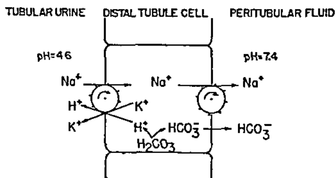
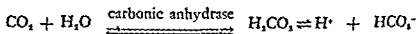



Fig. 14. Hypothetical mechanism of distal tubular and/or collecting duct cells concerned with reabsorption of sodium ions in exchange for hydrogen ions and potassium ions. (From R.F. Pitts. *Am. J. Med.*, 24 745, 1958)

These three facts *prompt us to postulate the existence of two* sodium pumps in the distal tubular cell as shown in Figure 14. One, located in the luminal membrane, is a coupled ion exchange pump, transporting sodium ions into the cell in exchange for either hydrogen or potassium ions. However, this pump transports more sodium in an inward direction than it does hydrogen and potas-

lines of evidence suggest that bicarbonate is reabsorbed indirectly, and that the mechanism involves hydrogen for sodium exchange coupled with carbon dioxide diffusion. Inhibitors of the enzyme carbonic anhydrase partially block bicarbonate reabsorption, implying that the reaction



$$CO_2 + H_2O \underset{}{\overset{\text{carbonic anhydrase}}{\rightleftharpoons}} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

is a significant step in the transport process. An increase in $pCO_2$ of the blood, which drives the reaction to the right and increases the hydrogen ion concentration within the tubular cells, facilitates the reabsorption of bicarbonate. Lowering the $pCO_2$ of the blood by hyperventilation inhibits reabsorption. Were bicarbonate reabsorbed as a stable ion species as is chloride, one would not expect transport to be affected by the above mentioned factors.

Some readers may object to the thesis outlined in Figure 13 on the basis of the low pH of the interior of the tubular cell. Although Boyle and Conway and Conway and Fearon maintain that the intracellular pH of muscle is 6.0, Wallace and Hastings and Gardner, MacLachlan and Berman claim it is between 6.4 and 6.9. Anderson and Mudge calculate that the pH of the interior of cells of renal cortical slices varies from 7.0 to 7.4. Actually it is not necessary for the pH of proximal cells to be as low as 6.1 for hydrogen ions to diffuse from cell contents to lumen.

If the transtubular potential is greater than 20 millivolts, the pH of the tubular cell could be higher than 6.1 and passive diffusion of hydrogen ions might still occur. The value of 20 millivolts for transtubular potential applies to the amphibian kidney; potentials as high as 39 millivolts have been recorded from proximal tubules of rats. It is possible, therefore, for the cell pH to be within a more reasonable range, yet passive diffusion of hydrogen ions might still occur. That transport is passive is far from proven.

Indeed recent observations of Gottschalk, Giebisch, and Windhager that the urine may be acidified in the proximal segment in osmotic diuresis suggest the possibility of active transport of hydrogen ions. Differences between proximal and distal ion transport mechanisms may be more quantitative than qualitative. How-

Hoeber had shown that acidification is depressed by sulfanilamide, an inhibitor of the enzyme carbonic anhydrase. Pitts and Alexander found that sulfanilamide markedly reduced the excretion of titratable acid by the acid and phosphate loaded dog. They interpreted these results in terms of the diagram presented in Figure 15A.

Alkaline dibasic sodium phosphate enters the renal tubule in the glomerular filtrate and is converted in the distal segment into acid monobasic sodium phosphate by the exchange of one hydrogen ion for one sodium ion per molecule of phosphate. The hydrogen ions are derived from carbonic acid, formed within distal tubular cells by the hydration of carbon dioxide, a reaction which is catalyzed by carbonic anhydrase. In the presence of enzyme inhibitors such as sulfanilamide, hydration of carbon dioxide to carbonic acid slows, and the rate at which hydrogen ions are presented to the exchange mechanism is reduced. The excretion of titratable acid is depressed. Under normal conditions, the hydrogen ions exchanged for sodium ions are excreted as monosodium-dihydrogen phosphate, i.e., as titratable acid. The sodium ions are restored to the blood stream along with equivalent numbers of bicarbonate ions. Pitts and Alexander pointed out that the exchange of hydrogen ions for sodium ions in the formation of acid urine is an energy consuming process. Figure 14 illustrates this fact in terms of a coupled ion pump located in the luminal membrane of the tubular cell and concerned with the active extrusion of hydrogen and the active reabsorption of sodium ions

*Exchange of Hydrogen Ions for Sodium Ions in the Distal Tubular Reabsorption of Sodium Bicarbonate* was proposed a year later by Pitts and Lotspeich. This concept is illustrated in Figure 15B. Under normal conditions, much more bicarbonate than phosphate enters the distal tubules. The exchange of hydrogen for sodium converts bicarbonate ions in the tubular urine into carbonic acid. This carbonic acid dehydrates to some extent to $CO_2$ and $H_2O$, and the $CO_2$ diffuses back across the tubular epithelium to enter the hydration cycle in the tubular cell. The sodium ions are reabsorbed along with equivalent numbers of bicarbonate ions. Distal reabsorption of bicarbonate like distal

sium in an outward direction. Chloride is reabsorbed in an amount sufficient to achieve ionic equivalence. It is possible that this reabsorption of chloride is passive, the ions moving downhill into the peritubular fluid along an electrochemical gradient in much the same fashion and driven by the same forces postulated in the proximal tubule. The movement of bicarbonate ions from the interior of the cell to the peritubular fluid may also be downhill along the existing potential gradient. A second pump, located in the peritubular membrane of the cell, ejects sodium from the cell, maintaining the intracellular sodium concentration at a low value. This pump like that of the proximal tubular cell originates the transcellular potential in some manner or other. It might be an electrogenic ion pump or it might be electrically neutral and transport potassium ions into the cell by the coupled carrier mechanism postulated earlier.

## OPERATION OF ION EXCHANGE PROCESSES IN THE DISTAL NEPHRON

An appreciation of the operation of distal tubular ion exchange in the regulation of acid-base balance and potassium metabolism is necessary for an understanding of the mechanism of diuresis induced by acidifying salts, potassium salts, and carbonic anhydrase inhibitors, and for elucidation of the potassium deficit which may result from intensive therapy with any diuretic. Normal operation of the ion exchange mechanisms will be considered briefly in the following paragraphs; alterations in their operation in diuretic therapy will be discussed in Chapters XI, XVII, XVIII and XIX.

*Evidence for Exchange of Hydrogen Ions for Sodium Ions in the Formation of Urinary Titratable Acid* was first presented by Pitts and Alexander in 1945. They observed that dogs, rendered acidotic by the administration of ammonium chloride and infused with large quantities of neutral sodium phosphate, excrete far more titratable acid in the urine than is present in the glomerular filtrate. The renal tubules must add hydrogen ions to the tubular urine. Earlier Montgomery and Pierce had observed that the urine is acidified in the distal segment of the amphibian nephron, and

buffer content of the urine is low. Briggs, Pitts, Berliner and others have proposed that ammonia diffuses passively from tubular cells into tubular urine down a gradient of hydrogen ion concentration. Walker first showed in the amphibian kidney that ammonia is secreted into the tubular urine at the site of acidification, a finding confirmed by Pitts et al in the mammalian kidney by means of the "stop-flow" technique described in Chapters XVI and XVII. If as shown in Figure 15C, only salts of strong non-buffer acids are present in the urine in appreciable quantities, essentially no titratable acid can be excreted, for the tubules can develop a hydrogen ion gradient no greater than 1,000:1 between urine and blood (urine pH, 4.4; blood pH, 7.4). Accordingly little sodium could be salvaged by an ion exchange process which resulted only in formation of titratable acid, for under normal conditions, the amount of fixed buffer available to neutralize hydrogen ions in the urine is relatively small. However, ammonia diffuses from tubular cells into acid urine as un-ionized $NH_3$, buffers $H^+$ ions to form $NH_4^+$ ions, and permits the continued exchange of $H^+$ for $Na^+$ ions. Ordinarily ammonia buffers two to three times the quantity of hydrogen ions buffered by phosphate and anions of other weak acids.

According to Van Slyke, Archibald and their associates, distal tubular cells contain an enzyme glutaminase which catalyzes the hydrolysis of glutamine to glutamic acid and ammonia. Most of the urinary ammonia is derived from glutamine, delivered to the distal tubular cells in the peritubular blood. A minor source of urinary ammonia is amino acids, oxidatively deaminized to the corresponding keto acids and ammonia. Cell membranes are relatively permeable to un-ionized $NH_3$, impermeable to $NH_4^+$ ions. Ammonia therefore diffuses from its site of formation within tubular cells to lumen, where it is trapped as ammonium ion. The actual transport of ammonia is passive and dependent on a gradient of concentration across the luminal membrane of the cell.

***Participation of Ion Exchange Mechanisms in Acid-Base Regulation.*** The three mechanisms described above participate in the regulation of acid-base balance in the following way. The usual mixed diet is acid ash; i.e., its content of fixed acid anions, chloride,

elaboration of titratable acid is depressed by carbonic anhydrase inhibitors. In fact the two mechanisms are one and the same. If little bicarbonate and much fixed buffer (phosphate) is present in distal tubular urine, the exchange of hydrogen ions for sodium ions results mainly in the formation of titratable acid. If much

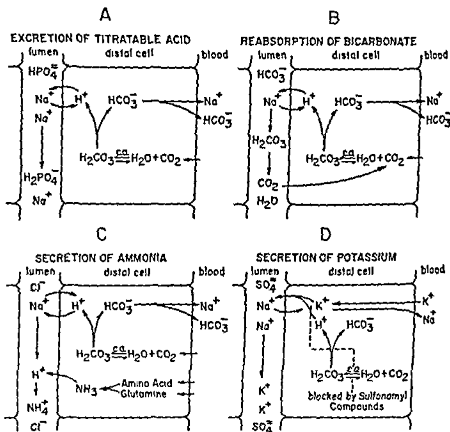



Fig. 15. The operation of the distal tubular and/or collecting duct ion exchange mechanism in the regulation of acid base balance and in the regulation of potassium content of extracellular fluid.

bicarbonate and little phosphate is present, the exchange of hydrogen ions for sodium ions results mainly in the reabsorption of bicarbonate.

*Buffering of Hydrogen Ions by Ammonia* permits continued exchange of hydrogen ions for sodium ions even though the fixed

exchanged for sodium ions, body stores of potassium are depleted and acidosis results.

Under normal conditions some 80 per cent of the filtered potassium is reabsorbed, only 20 per cent is excreted (see Table IV). Berliner believes that all of the filtered potassium is reabsorbed in the proximal tubule and that the moiety excreted is derived entirely from that secreted by the distal tubules in exchange for sodium. The evidence for this statement is by no means conclusive.

Ullrich and his associates have recently demonstrated by the ingenious technique of catheterizing the orfices of collecting ducts with filamentous polyethylene catheters that hydrogen-sodium exchange and ammonia secretion are functions of the collecting ducts as well as of the distal convoluted tubules. One should therefore describe these mechanisms as located in the distal nephron, not as previously, in the distal convoluted tubule. "Stop-flow" studies are consonant with this view.

*Electron Microscopic Studies of Renal Tubules* have demonstrated an amazing complexity and diversity of structure in the several segments of the nephron. Unfortunately, our understanding of both structure and function is at present too inadequate to permit the formulation of any very significant correlations between the two. However, to a physiologist, the following morphological features seem especially pertinent to an understanding of function.

First, the areas of the luminal and basal surfaces of tubular cells are tremendously increased by a complicated series of evaginations and invaginations of the limiting plasma membrane (see Fig. 16). In the proximal segment, the luminal surface of the cell is densely populated with cylindrical fingerlike evaginations, called microvilli, the brush border of light microscopists. These processes average 10,000 Å (1 micron) in length and 700 Å in diameter. They are covered with a thin plasma membrane and are otherwise structureless. In the conventionally fixed specimen, they are closely packed, some 215 per square micron, although in life with the tubule distended, they are no doubt separate and free floating in the tubular fluid. Tiny coiled ducts, which open at the

phosphate, and sulfate exceeds its content of fixed cations, sodium, potassium, calcium, and magnesium. Stated in another way, the normal diet imposes a fixed acid load on the body, a load which is neutralized by buffers of the body fluids, largely bicarbonate. If the acid anions were excreted as sodium salts, body reserves of buffer would be depleted and body fluids would become acid. Actually sodium is conserved, and the excess of anions is excreted either as titratable acid (mechanism A of Fig. 15) or in combination with ammonia (mechanism C of Fig. 15). The filtered sodium bicarbonate which escapes reabsorption in the proximal segment is completely removed from the distal urine (mechanism B of Fig. 15).

*Exchange of Potassium Ions for Sodium Ions* is involved in the regulation of acid-base balance and in the regulation of extracellular concentration and, indirectly, total body content of potassium. The exchange mechanism was first described by Berliner, Gilman and Mudge. Because they early recognized that hydrogen ions and potassium ions compete in exchange for sodium, the mechanism was assigned to the distal tubule. Confirmation of distal localization in the nephron of the dog has been provided by studies utilizing the "stop-flow" technique. Figure 15D illustrates the major characteristics of potassium exchange. If cellular and extracellular reserves of potassium are increased by the administration of potassium salts, potassium ions rather than hydrogen ions are exchanged for sodium. Failure to excrete hydrogen ions results in the development of hyperkalemic metabolic acidosis, usually of minor proportions. If cellular and extracellular reserves of potassium are depleted due to inadequate intake or excessive loss, hydrogen rather than potassium ions are exchanged for sodium. Enhanced excretion of hydrogen ions results in the development of hypokalemic metabolic alkalosis, a form of alkalosis resistant to treatment except by correction of the potassium deficit. Adrenal steroids enhance the overall activity of the exchange mechanism, thus increase both hydrogen and potassium exchange. Steroid excess therefore induces both hypokalemia and alkalosis. Inhibitors of carbonic anhydrase reduce the supply of hydrogen ions to the exchange mechanism. Potassium rather than hydrogen ions are

The basilar surfaces of both proximal and distal tubular cells are extensively infolded to form what are called cytoplasmic lamellae by Rhodin. These lamellae penetrate more or less deeply into the tubular cell (see Fig. 16) to divide its basal portion into a series of narrow open ended compartments in which mitochondria are linearly arranged. The basement membrane follows these invaginations, forming a double walled slit in open communication with the peritubular space. The slits are extensively branched and interconnected and tremendously increase the area of the basal surface of the cell. In the proximal segment and collecting ducts, the slits penetrate less deeply into the soma of the cells and the mitochondria are less obviously aligned with the surface invaginations than in distal cells. One might theorize that the arrangement in distal tubular cells would especially favor active pumping of ions. Mitochondria adjacent to the surface invaginations could supply phosphate bond energy to drive ion pumps in the membrane. Perhaps the alignment of mitochondria and slits is more evident in distal cells because of the greater energy expenditure demanded by the development of ion gradients in this segment.

A second feature of some significance is the delicate structure of peritubular capillaries. These vessels consist of a very thin fenestrated endothelial layer and a thin basement membrane. They are closely applied to the tubular cells. The system appears to be one which would permit the ready transfer of water and solutes between tubular cells and blood stream.

A third feature of note are the specializations to form a "tight tube." In proximal and distal tubules trabecular processes extend out radially from the basal portions of the cells. These trabeculae insert under adjacent cells and interlock with trabeculae of those cells. In the thin segment of the loop of Henle, where the cells are much attenuated, the cell borders are serrated and enmesh as a series of gear teeth. or more appropriately as pieces of a jig saw puzzle. Throughout the tubule, the chinks between cells are sealed at the luminal surface by terminal bars The arrangement would appear to be one to ensure that whatever crosses the tubular epithelium in either direction does so by transfer through the cell. Leakage between cells must be minimal. The correlations of

bases of the microvilli, penetrate into the soma of proximal cells. Some of these ducts connect with cytoplasmic vacuoles. Microvilli and microducts are most numerous in the convoluted portion of the proximal tubule. They diminish in number in the thick descending portion of Henle's loop, and are present only in rudimentary form



Fig. 16. Schematic representation of the structure of a proximal tubular cell as revealed by the electron microscope. (From J. Rhodin: Thesis, Stockholm, 1954, Karolinska Institute)

in the thin segment of the loop, distal tubule and collecting duct. These structures greatly increase the luminal surface available for diffusion, in fact for exchanges of any type between cell and tubular fluid. In view of the large quantities of water, sodium, chloride and hydrogen ions transferred across the luminal borders of proximal tubular cells, microvilli and microducts might be considered as rather simple but effective specializations increasing surface and therefore favoring bulk transfer.

The basilar surfaces of both proximal and distal tubular cells are extensively infolded to form what are called cytoplasmic lamellae by Rhodin. These lamellae penetrate more or less deeply into the tubular cell (see Fig. 16) to divide its basal portion into a series of narrow open ended compartments in which mitochondria are linearly arranged. The basement membrane follows these invaginations, forming a double walled slit in open communication with the peritubular space. The slits are extensively branched and interconnected and tremendously increase the area of the basal surface of the cell. In the proximal segment and collecting ducts, the slits penetrate less deeply into the soma of the cells and the mitochondria are less obviously aligned with the surface invaginations than in distal cells. One might theorize that the arrangement in distal tubular cells would especially favor active pumping of ions. Mitochondria adjacent to the surface invaginations could supply phosphate bond energy to drive ion pumps in the membrane. Perhaps the alignment of mitochondria and slits is more evident in distal cells because of the greater energy expenditure demanded by the development of ion gradients in this segment.

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structure and function outlined above must be considered only as speculations.

## SUMMARY

Urine formation begins with the ultrafiltration of large volumes of plasma through the porous walls of the glomerular capillary tufts. The glomerular ultrafiltrate contains all crystalloidal components in essentially the same concentrations as exist in the aqueous phase of the plasma from which it is formed. To prevent rapid exhaustion of limited body reserves, the bulk of the filtered water and of the filtered sodium, chloride, and bicarbonate ions is reabsorbed by the renal tubules; less than 1 per cent is normally excreted. It is possible to account for a number of these reabsorptive functions in terms of the active tubular transport of a single ion species, sodium.

It is probable that sodium pumps, located in the peritubular membrane of proximal tubular cells, account for the active reabsorption of the major fraction (4/5ths to 7/8ths) of the filtered sodium. The electrical forces set up by such pumps may cause the passive migration of chloride ions into the blood stream along a favorable electrical gradient. Bicarbonate ions may be reabsorbed indirectly in the proximal segment by the passive migration of hydrogen ions from tubular cell to tubular lumen. The osmotic force created by the active reabsorption of ions and other solutes accounts for the passive transfer of an equivalent proportion of the water.

The conditions governing ion reabsorption in the loops of Henle, in the distal tubules, and in the collecting ducts are more demanding. Concentration gradients developed for sodium and hydrogen ions and the interrelations of sodium, hydrogen and potassium transport suggest that the reabsorptive mechanisms in the distal parts of the nephron are more complicated. Perhaps two types of sodium pumps are necessary, one type located in the luminal membrane, the other in the peritubular membrane. Both may be coupled ion pumps.

The elaboration of urine hypertonic to the blood plasma, a function now generally conceded to reside in the collecting ducts, need not involve the active transport of water. The active

pumping of sodium from the ascending limbs of the loops of Henle into the interstitium of the medulla and papilla renders the tissue hypertonic. Water moves from the collecting ducts into the interstitium by osmosis; the osmolal concentration of the final urine is essentially the same as that of papillary tissue.

## REFERENCES


1. Bayliss, L. E.· The process of secretion, in *Modern Views On The Secretion of Urine*, F. R. Winton, Ed., London, J. & A. Churchill, Ltd, 1956.
2. Berliner, R. W., Levinsky, N. G., Davidson, D. G., and Eden, M. Dilution and concentration of the urine and action of antidiureitc hormone. *Am. J. Med.*, *24*:730, 1958.
3. Giebisch, G.: Electrical potential measurements on single nephrons of Necturus. *J. Cell. Physiol.*, *51*:221, 1958.
4. Gottschalk, C. W. and Mylle, M.: Micropuncture study of the mammalian urinary concentrating mechanism· evidence that the loop of Henle participates in a counter-current multiplier system. *Am. J. Physiol.*, *196*:927, 1959.
5. Hilger, H. H., Klumper, J. D and Ullrich, K. J.: Wasserruckresorption und Ionentransport durch die Sammelrohrzellen der Saugetierniere. *Pflugers Arch*, *267*:218, 1958.
6. Hoshiko, T., Swanson, R. E., and Visscher, M. B. Excretion of $Na^{22}$ and $K^{42}$ by the perfused bullfrog kidney and the effect of some poisons. *Am. J. Physiol*, *181*:542, 1956.
7. Malvin, R. L., Wilde, W. S., Vander, A J., and Sullivan, L. P.: Localization and characterization of sodium transport along the renal tubule. *Am. J. Physiol*, *195*·549, 1958.
8. Pappenheimer, J. R. Über die Permeabilitat der Glomerulummembranen in der Niere. *Klin. Wchnschr*, *33*:362, 1955.
9. Pease, D. C. Fine structure of the kidney seen by electron microscopy. *J. Histochem. & Cytochem.*, *3*:295, 1955.
10. Pitts, R. F.: Acid-base regulation by the kidneys. *Am. J. Med.*, *9*:356, 1950.
11. Pitts, R. F.: Some reflections on mechanisms of action of diuretics *Am. J Med.*, *24* 745, 1958.
12. Pitts, R. F., Gurd, R. S., Kessler, R. H., and Hierholzer, K.. Localization of acidification of urine, potassium and ammonia secretion and phosphate reabsorption in the nephron of the dog. *Am J. Physiol.*, *194*:125, 1958.

13. RICHARDS, A. N.: Urine formation in the amphibian kidney. *Harvey Lect.*, *30*:93, 1934-35.
14. RHODIN, J.: Electron microscopy of the kidney. *Am. J. Med.*, *24*: 661, 1958.
15. SELKURT, E. E.: Sodium excretion by the mammalian kidney. *Physiol. Rev.*, *34*:287, 1954.
16. SMITH, H. W.: *Principles of Renal Physiology*. New York, Oxford Press, 1956.
17. USSING, H. H.: Active transport of inorganic ions. Active Transport and Secretion. VIII. *Symposia of the Society for Experimental Biology*. New York, Academic Press, 1954, p. 407.
18. WALKER, A. M., BOTT, P. A., OLIVER, J., and MACDOWELL, M. C.: The collection and analysis of fluid from single nephrons of the mammalian kidney. *Am. J. Physiol.*, *134*:580, 1941.
19. WESSON, L. G. Glomerular and tubular factors in the renal excretion of sodium chloride. *Medicine*, *36*:281, 1957.
20. WINDHAGER, E. E., SCHATZMANN, H. J., WHITTEMBURY, G., and SOLOMON, A. K.: Studies in single proximal tubules of the Necturus kidney. III. The effect of transtubular sodium concentration gradients or of osmotic gradients on water movement. *Am. J. Physiol.*, to be published.
21. WIRZ, H.· The production of hypertonic urine by the mammalian kidney. *Ciba Symposium on the Kidney*. London, J. &. A. Churchill, Ltd., 1954, p. 38.
22. WIRZ, H.: The location of antidiuretic action in the mammalian kidney. The Neurohypophysis. *Proceedings of the VIII Symposium of the Colston Research Society*. New York, Academic Press, 1957, p. 157.

*Chapter V*

# REGULATION OF VOLUME AND OSMOLAL CONCENTRATION OF EXTRACELLULAR FLUID

THE volume of extracellular fluid, as reflected in the body weight of a normal adult in caloric balance, is held remarkably constant from day to day despite variations in intake of salt and water. The osmolality of extracellular fluid is stablized with even greater precision. Within limits, volume and osmolality are regulated independently; *however under stress, the regulatory mechanisms* interact, and precise regulation of one variable may be sacrificed to permit some control of the other. A volume receptor-renal effector mechanism governs the rate of excretion of salt, thus the total salt content and hence the volume of the extracellular compartment. Actually the complete regulatory mechanism must involve salt appetite as well, but since the average diet contains more than enough electrolyte to satisfy needs, one ordinarily overlooks this element of the system. An osmoreceptor-renal effector mechanism governs the rate of excretion of water, and in association with thirst, regulates the osmolality of the extracellular fluid.

## REGULATION OF EXTRACELLULAR VOLUME

*The Volume Receptor-renal Effector Complex* is no doubt a *natriuretic-antinatriuretic mechanism; i.e. it basically regulates the* excretion of sodium. While affected by, it is not primarily responsive to the concentration of sodium. Rather it is responsive to the volume of extracellular fluid; to some fraction of that volume, such as that of plasma or interstitial fluid, to some derivative of volume, such as intra-vascular or interstitial pressure; or perhaps to blood flow. It is probable that the system consists of a

13. RICHARDS, A. N.: Urine formation in the amphibian kidney. *Harvey Lect.*, *30*:93, 1934-35.
14. RHODIN, J.: Electron microscopy of the kidney. *Am. J. Med.*, *24*: 661, 1958.
15. SELKURT, E. E.: Sodium excretion by the mammalian kidney. *Physiol. Rev.*, *34*:287, 1954.
16. SMITH, H. W.: *Principles of Renal Physiology*. New York, Oxford Press, 1956.
17. USSING, H. H.: Active transport of inorganic ions. Active Transport and Secretion. VIII. *Symposia of the Society for Experimental Biology*. New York, Academic Press, 1954, p. 407.
18. WALKER, A. M., BOTT, P. A., OLIVER, J., and MACDOWELL, M. C.: The collection and analysis of fluid from single nephrons of the mammalian kidney. *Am. J. Physiol.*, *134*:580, 1941.
19. WESSON, L. G.: Glomerular and tubular factors in the renal excretion of sodium chloride. *Medicine*, *36*:281, 1957.
20. WINDHAGER, E. E., SCHATZMANN, H. J., WHITTEMBURY, G., and SOLOMON, A. K.: Studies in single proximal tubules of the Necturus kidney. III. The effect of transtubular sodium concentration gradients or of osmotic gradients on water movement. *Am. J. Physiol.*, to be published.
21. WIRZ, H.: The production of hypertonic urine by the mammalian kidney. *Ciba Symposium on the Kidney*. London, J. &. A. Churchill, Ltd., 1954, p. 38.
22. WIRZ, H.: The location of antidiuretic action in the mammalian kidney. The Neurohypophysis. *Proceedings of the VIII Symposium of the Colston Research Society*. New York, Academic Press, 1957, p. 157.

tonicity of medullary and papillary tissue, thus with the elaboration of concentrated urine; and a distal tubule-collecting duct mechanism, concerned with acid base regulation and potassium excretion, with the elaboration of salt-poor dilute urine, and with the fine regulation of sodium balance.

The rate at which sodium is reabsorbed by the proximal mechanism varies as some function of glomerular filtration rate, but to date it has been impossible to describe the function precisely. According to Walker and his associates, some 12.5 per cent of the fraction of sodium remaining in the proximal segment is reabsorbed in each succeeding 10 per cent of tubule length. According to Smith, roughly seven-eights of the filtered sodium is reabsorbed at all levels of filtration rate. Suffice it to say that if filtration rate increases, the absolute amount of sodium reabsorbed in the proximal segment increases; if filtration rate decreases, the absolute amount of sodium reabsorbed decreases. However, of more immediate significance for the maintenance of sodium balance is the fact that an increase in filtration rate results in the delivery of more sodium into distal parts of the nephron; a decrease in filtration rate results in the delivery of less sodium. If, as seems probable, the reabsorptive capacities of these distal segments are limited, the rate of excretion of sodium will vary directly as some function of filtration rate.

The mechanism of the loop of Henle probably reabsorbs at least 5 per cent of the filtered sodium. This statement derives from the fact that some 4 to 5 ml. of solute free water are reabsorbed in the collecting ducts in the final transformation of isotonic tubular fluid into maximally concentrated urine. The motive force for this reabsorption of water is the hyperosmolality of medullary and papillary tissue created by the reabsorption of sodium in the loops of Henle. The quantity of water which can be reabsorbed in the collecting ducts is obviously related to the quantity of sodium reabsorbed by the ascending limbs of the loops of Henle.

Under normal conditions the distal tubule-collecting duct mechanism reabsorbs some 10 to 15 per cent of the filtered sodium. This mechanism is a heterogenous one, engaged in the reabsorption of sodium and chloride as ion pairs and in the reabsorption of

receptor organ which senses volume, pressure, or flow, a hypothalamic integrative center, and an efferent neuro-humoral regulatory mechanism which operates through the kidney to control the rate of excretion of salt. In no wise is any part of this system as clearly and adequately defined as is the corresponding part of the osmo-regulatory mechanism. Much of what can be said must be considered as speculative.

*Volume Receptor Organs.* As one might gather from the statements above, *both the nature of the stimulus and the locus* and nature of the receptor endings are in dispute. Harrison and Strauss maintain that the receptor mechanism is in the cephalad portion of the body and that it is either directly sensitive to the extracellular volume or to its derivatives, venous pressure or distension. Epstein on the other hand suggests that the receptor mechanism is in the arterial reservoir and that the degree of distension of this reservoir generally, or the distension of some highly sensitive portion of it, initiates the afferent messages which ultimately modulate salt excretion. Borst, in contrast, believes that the receptor mechanism is sensitive to variations in cardiac output or perhaps to cardiac output relative to metabolic demands of the body.

*Integrative Mechanism.* The locus of the integrative mechanism is unknown, but it is logical to assume that it is in the hypothalamus for the following reasons. Although afferent impulses for the most part are received from volume receptors of unknown location, efferent outflow of the integrative center is directed to hypothalamic and cortical centers for thirst and salt craving; to hypothalamic autonomic centers controlling renal hemodynamics, to a postulated diencephalic center controlling aldosterone secretion, and to the hypothalamic center for osmoregulation. It is likely that the integrative center which serves to coordinate the activities of these several mechanisms is also in the hypothalamus.

*Renal Effector Mechanism.* Mechanisms of reabsorption of sodium have been described in Chapter IV. Broadly speaking, they fall into three categories: A proximal mechanism, concerned with the reabsorption of the bulk of the filtered sodium; a mechanism in the loop of Henle, concerned with the maintenance of hyper-

constancy of extracellular fluid volume. (1) If salt intake increases, extracellular volume expands; volume receptors are stimulated. Nerve mesasges relayed through the integrative center, a hypothalamic autonomic center and renal vasomotor nerves, increase renal blood flow and glomerular filtration rate. More sodium is delivered to the reabsorptive mechanism of the distal tubules and collecting ducts; more escapes reabsorption and is excreted. The inverse sequence, initiated by a decrease in extracellular volume, leads to reduced filtration, to over-reabsorption, and to diminished excretion of sodium. As will be evident below, this mechanism plays a prominent role in the dog. (2) Expansion of extracellular volume leads via the integrative center and the diencephalic center regulating aldosterone secretion, to reduced secretion of salt retaining steroid. Reabsorption of sodium in the distal tubules and collecting ducts diminishes and excretion increases. The inverse sequence, initiated by a decrease in extracellular volume, results in increased reabsorption and diminished excretion of sodium. As will be evident below, this mechanism plays a prominent role in man.

***Response to Salt Loading.*** Normal man tolerates variations in salt intake within a range of 1 to 10 gm. per day with minimal fluctuations in body weight. However, if some 30 or 40 gm. of salt are added to the diet each day, body weight increases promptly and stabilizes at a value some 5 to 15 lb. above normal. This increase in body weight is due to expansion of extracellular fluid volume, i.e., to the collection of occult and even overt edema. As Luetscher, Bartter and others have shown, the rate of urinary excretion of salt retaining steroids is markedly diminished or indeed abolished under such conditions. Stabilization of the volume of extracellular fluid at a moderately elevated level instead of progressive sodium retention is no doubt made possible largely by abolition of aldosterone stimulation of a small but highly significant fraction of salt reabsorption. According to Leaf *et al.*, Green *et al.*, and Crawford and Ludemann, salt loading causes relatively little increase in glomerular filtration rate in normal man. This no doubt underlies his relative intolerance of high dietary salt intake and his very sluggish excretion of an intravenous load of saline.

sodium in exchange for hydrogen, ammonia, and potassium. Overall reabsorptive capacity is limited. Hence if the mechanism is presented with an excess of sodium in consequence of increased filtration rate, reabsorptive capacity is exceeded and excretion increases; if presented with less sodium, all may be reabsorbed. The transport capacity of the distal tubule-collecting duct mechanism is enhanced by aldosterone, but the steriod sensitive fraction of reabsorption is probably small. This latter statement derives from the fact that the adrenalectomized dog or man excretes only 2 per cent or so of the filtered sodium (see Chapter VI). It is possible that the aldosterone sensitive fraction of reabsorption is greater than 2 per cent, but that in the absence of hormone, other regulatory mechanisms compensate for most of the reabsorptive deficit. In any event, hormonal control of a final, albeit small, fraction of tubular reabsorption provides powerful leverage in the control of sodium balance.

If a small fraction of sodium reabsorption fails due to lack of circulating hormone (adrenalectomy, Addison s disease), body stores of sodium, chloride, and water are gradually depleted. Furthermore, because of reduced exchange of hydrogen, potassium and ammonia for sodium, hyperkalemia and metabolic acidosis develop. In contrast, when excessive amounts of aldosterone are secreted, hydrogen, ammonia, and potassium are exchanged for sodium in increased amounts. Hypokalemia and metabolic alkalosis develop and sodium, chloride, and water are retained. However the relationship is not a simple one. Primary hyperaldosteronism, due to glandular hyperplasia or adenoma, is characterized more by hypokalemia and alkalosis than by sodium retention. In contrast, secondary hyperaldosteronism, now recognized as occurring in variable degree in all states of active accumulation of edema, is characterized more by sodium retention than by hypokalemia and alkalosis. Other unrecognized factors must be significent in determining ion balances.

*Mode of Action of Neurohumoral Regulatory Mechanisms.* Given a series of reabsorptive mechanisms with the diverse properties described above, it is reasonable to assume that sodium output may be balanced against intake in two ways to achieve

it must contribute to glomerulo-tubular imbalance and to salt retention (see Chapter VI on renal factors in the formation of edema). However, a reduction in glomerular filtration rate is by no means the whole story. Goodyer and Jaeger have proposed an interesting hypothesis in explanation of immediate reduction of salt excretion on erect standing in subjects exhibiting no gross change in filtration rate. They propose that erect standing favors the formation of an increased proportion of filtrate in long, high salt absorbing nephrons and of a decreased proportion of filtrate in short salt wasting nephrons Unfortunately, there is no evidence in favor of this thesis other than the well documented observation of salt and water retention under conditions which lead to general compensatory alterations in hemodynamics. Most investigators have preferred to emphasize increased tubular reabsorption of salt and water, stimulated respectively by increased secretion of aldosterone and of antidiuretic hormone. In favor of this thesis is the observation of Muller and others of a diurnal variation in rate of excretion of aldosterone in the urine of normal man: high during the day (orthostasia), low at night (recumbency). Also favorable is the finding of Davis, Farrell and others that the rate of liberation of aldosterone into adrenal venous blood of the dog increases progressively during hemorrhage.

Two facts make it difficult to explain compensatory salt retention in man entirely in terms of increased secretion of aldosterone. First, retention of salt in response to erect standing, bleeding, or trapping of blood in the limbs is prompt; adrenal steroids given intravenously stimulate salt reabsorption after a latent period of 40 min. to an hour or more. Second, Rosenbaum et al have shown that patients with Addison's disease, on maintenance doses of adrenal steroids, exhibit a normal renal salt conserving response to bleeding, assumption of erect posture and compression of the thighs.

It seems evident that no single mechanism can explain salt retention under all circumstances of depletion of extracellular volume. Relative over-reabsorption from a reduced volume of filtrate, redistribution of filtrate among nephrons of diverse function, oversecretion of aldosterone and perhaps other as yet

In contrast the volume receptor-renal effector mechanism of the dog responds briskly and effectively to salt loading. Ladd and Raiz have shown that the dog can tolerate as much as 4 gm. of salt per Kg. per day for long periods of time, gaining weight with water ingestion after each meal, losing it overnight. Per Kg. of body weight, this quantity of salt would correspond to a load of 280 to 300 gm. per day in man, a value far in excess of any that can be tolerated. Green and Faragh, Wesson and Anslow, Mudge and others have shown that the rate of glomerular filtration of the dog increases promptly on salt loading by as much as 100 per cent and that, for brief periods, the rate of excretion of sodium may attain the phenomenal value of 40 per cent of that filtered. It is obvious that normal man, but not the normal dog, is only a "salt shaker" away from incipient edema, and that stability of filtration rate in man at least partially explains his intolerance of salt loading in comparison with the dog. It is understandable why even mild reduction of glomerular filtration rate or even moderate elevation of aldosterone production in cardiac, hepatic or renal disease may lead to manifest edema. Just why the dog should be so thoroughly protected against the stress of salt loading and man should be so vulnerable, is a mystery.

***Response to Volume Depletion.*** While the homeostatic response of normal man to expansion of extracellular volume compares unfavorably with that of the dog, his response to the more immediately vital threat of volume reduction is prompt and effective. Goodyer, Seldin, Brun, Farber, Epstein and others have shown that urine flow and salt excretion drop precipitously when a relatively small proportion of the circulating blood volume is sequestered by venous tourniquets on the thighs, is redistributed by erect standing or by opening an arterio-venous fistula, or is removed by phlebotomy. All three procedures excite the sensation of thirst. While the response is obviously of homeostatic significance in combating an actual or apparent reduction of extracellular volume, the responsible mechanisms have by no means been adequately defined. Glomerular filtration rate has been variously described as decreasing moderately, decreasing equivocally, and exhibiting no change. If filtration rate decreases, it is evident that

It is apparent from recent evidence that ACTH does exert some influence over aldosterone production, injection of ACTH increasing, hypophysectomy decreasing glandular secretion of salt active materials. However, the effects of ACTH or hypophysectomy on aldosterone production are far less marked than on glucocorticoid production. The basic conclusions drawn from the work of Deane and Greep are still valid.

Farrell and his associates have recently observed that decorticate, but not decerebrate or decapitate animals secrete normal quantities of aldosterone into adrenal venous blood, an observation which suggests that a diencephalic neurosecretory mechanism, independent of the hypophysis, controls hormone production. In support of this view, they have observed that injection of extracts prepared from the diencephalon causes the discharge of aldosterone into the adrenal veins. At a recent Lauretian conference they reported that an acid extract of the subpineal region of the posterior diencephalon is most active in stimulating aldosterone secretion. They draw an analogy between the neurosecretory mechanism of the supraopticohypophyseal system which controls the renal reabsorption of water and that of the diencephalic-adrenal system which controls the renal reabsorption of salt. If their observations are confirmed, the nature of the neurohumoral control of aldosterone secretion will have been considerably advanced.

*Interaction of Volume and Osmoregulatory Mechanisms.* Patients in congestive failure who are subjected to vigorous diuretic therapy or those with cirrhosis from whom large volumes of ascitic fluid are removed by paracentesis may respond to such acute reductions of extracellular or transcellular fluid volume by vigorous retention of both salt and water. They experience marked thirst, and if water intake is unrestricted, may dilute their body fluids to a degree sufficient to produce signs and symptoms of severe water intoxication. They do not exhibit a normal water diuresis although their body fluids are markedly hypotonic. A somewhat similar syndrome occurs in otherwise normal individuals who sweat profusely. Sweating depletes the body of both salt and water and reduces extracellular volume. Replacement of water causes dilution, muscular cramping, and weakness. This syndrome suggests that

unrecognized mechanisms may contribute to the response. The difficulty of assessing the significance of small changes in filtration rate versus small changes in tubular reabsorptive activity in explanation of the retention of salt should be apparent to anyone who reflects for a moment on the facts that the total daily variation in excretion is commonly less than ± 170 mEq. of sodium; that the total filtered load is roughly 24,000 mEq., that the change in reabsorption is from 99 to 99.9 per cent of the filtered load; and that reabsorption is effected, not by a single mechanism, but by a variety of mechanisms of diverse properties distributed serially along the nephron. The final difficulty is that the measurement of filtered load is at best uncertain to ± 2 per cent.

*Site and Nature of Neuro-humoral Regulatory Mechanisms.* During the latter part of the last Century, piqure of the bulb, thalamus and hypothalamus were variously described as producing urinary salt wasting and hypochloremia, or urinary salt retention and hyperchloremia. Welt and his associates have more recently described a series of patients with diffuse central nervous system disease exhibiting a salt wasting syndrome, associated with low serum sodium and chloride. The evidence suggests that the central nervous system is in some way involved in the renal regulation of electrolyte balance, *yet it clarifies neither the locus nor the mode* of action of the neural structures involved.

It has long been known that the hypophysectomized animal or the patient with pan-hypopituitarism exhibits gross evidence of multiple endocrine deficiencies, including those of glucocorticoid deprivation. Equally clear has been the fact that the hypopituitary organism does not suffer from the severe electrolyte disturbances which are associated with adrenal insufficiency. The observation of Deane and Greep that the zona glomerulosa of the adrenal of the hypophysectomized animal retains its integrity, suggests that aldosterone is formed in this portion of the adrenal cortex, that the functional integrity of the aldosterone producing cells is independent of hypophyseal trophic hormones, and that the rate of secretion of aldosterone is regulated in accord with the needs of the body by some hormone other than adrenocorticotrophic hormone (ACTH).

the osmolal concentration falls. Shrinkage is thought to stimulate the processes of neurons of the supraoptic nucleus applied to the surfaces of the vesicles. Stimulation of these neurons leads to the secretion of antidiuretic hormone. Inhibition of these neurons by swelling of the vesicles stops hormone production and that which circulates is gradually destroyed by the tissues.

The injection of hypertonic solutions of sodium chloride into exteriorized carotid artery loops of trained dogs results in the prompt inhibition of water diuresis. The effect is produced by a small dose, calculated to increase the osmolality of the carotid blood by only two per cent. The injection of a much larger dose into a peripheral vein where it is diluted in a large volume of blood before reaching the receptor area has no effect on diuresis. The injection of urea into the carotid loop, a substance to which the vesicles are apparently freely permeable, has no effect on water diuresis; i.e., it exerts no osmotic effect to cause shrinkage of osmoreceptor vesicles.

*Hypothalamic Integrative Mechanism.* While the osmolal concentration of the blood plasma acting through osmoreceptors in the brain basically regulates the secretion of antidiuretic hormone, a variety of agents and stimuli exerts a subsidiary control. Antidiuresis due to liberation of antidiuretic hormone results from painful stimuli, exercise, syncope, smoking and from the administration of such drugs as nicotine, adenosine tri-phosphate, acetylcholine, epinephrine and histamine. Anesthetics, especially ether, morphine and the barbiturates are potent stimulators of antidiuretic hormone secretion. On the other hand, the release of antidiuretic hormone is supressed and diuresis results when the left atrium is distended by a balloon, when the suggestion of water drinking is made under hypnosis, as a result of establishment of conditioned reflexes and from the administration of alcohol These several excitatory and inhibitory stimuli are presumably integrated in a hypothalamic center, but whether in the supraoptic or paraventricular nuclei or in the adjacent hypothalamic tegmentum is unknown.

*Neurosecretory Mechanism.* The view that the antidiuretic hormone is actually formed in the cells of the supraoptic and para-

the volume regulatory center exerts an ancillary control over the osmoregulatory mechanism. If volume is markedly reduced, the antidiuretic mechanism is maximally engaged, even though the body fluids are excessively diluted. Volume is partially restored at the expense of a reduction in osmolality.

## REGULATION OF OSMOLAL CONCENTRATION OF BODY FLUIDS

Given free access to water and a diet of even minimal salt content, the normal individual regulates the osmolal concentration of his body fluids with remarkable precision. Normal limits are usually given as 285 and 310 mOsm. per liter. However, any one individual exhibits even greater constancy, varying only one or *two per cent from his characteristic mean. Osmolality is* regulated by the variable excretion of water in relation to osmotically active solutes. When the body is diluted by the ingestion of large quantities of water, diuresis of hypotonic urine restores the osmolal concentration of the body fluids to normal. When the body is concentrated by loss of water or by gain of solute, oliguria restricts further water loss; the formation of hypertonic urine permits the excretion of solute without the loss of equivalent amounts of water; and thirst drives the individual to restore his water deficit. The regulatory mechanism consists of an osmoreceptor system, a hypothalamic integrative mechanism, a neurosecretory mechanism producing antidiuretic hormone and a renal effector mechanism which governs the excretion of water. In contrast to the sluggish regulation of volume when an individual is salt loaded, osmolal concentration is promptly restored to normal when he is water loaded.

*Osmoreceptor Mechanism.* The elegant studies of Verney have demonstrated that receptors, sensitive to small changes in osmolality of the blood plasma perfusing them, are located bilaterally within the zones of distribution of the internal carotid arteries. He has suggested that small vesicles in the supraoptic nuclei of the hypothalamus may be the osmoreceptors. These vesicles are presumed to act as minute osmometers, to shrink when the osmolal concentration of their fluid environment rises, and to swell when

quarter hour thereafter takes an amount equivalent to the volume of urine excreted, he achieves and maintains a maximal state of hydration. Urine flow increases within an hour to 13 to 26 ml, per min., i.e. to a level which is maximal for that individual and which cannot be increased by more rigorous hydration procedures. If more water is ingested, it usually leads to nausea and vomiting or to diarrhea, not as might be presumed to water intoxication, to which the normal adult is remarkably resistant. The child or the patient with renal, hepatic, adrenal, or cardiac disease is considerably less tolerant of water and exhibits water intoxication more readily.

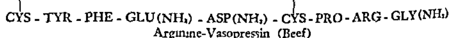
Hydration of this magnitude dilutes the body and reduces the osmolality of the body fluids by 3 to 5 per cent. The secretion of antidiuretic hormone is suppressed, that hormone which normally circulates is metabolized, and a state of physiological diabetes insipidus develops. A polyuria of dilute urine results. Nearly pure water is excreted in defense of the osmotic pressure of the body fluids.

When water intake is terminated, urine flow remains high for a time and the excess water in the body is eliminated. As the osmolality of the body fluids approaches its normal level, osmoreceptors are again stimulated, antidiuretic hormone secretion begins, and urine flow is gradually reduced to more normal values.

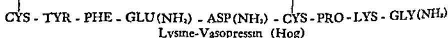
If fluid is withheld for a period of time, the osmolality of the body fluids rises as water is lost by the insensible routes of cutaneous and pulmonary evaporation and by urine formation. The osmoreceptors are stimulated to a greater than normal degree and antidiuretic hormone secretion increases above its basal rate Urine flow is reduced from its usual value of 1 to 2 ml. per min. to 0.5 ml. or less. The urine is highly concentrated, the solutes ordinarily excreted in 2 ml. of urine are now contained in 0.5 ml. or less. The osmolal concentration of the urine may increase to a value some 4 to 5 times that of the plasma. Obviously for each 0.5 ml. of urine excreted with an osmotic pressure 4 times that of plasma, the body gains 1.5 ml. of free water to expend in insensible evaporation or to dilute the body fluids. However, in a quantitative sense, the osmoreceptor-renal effector mechanism is far more

ventricular nuclei and transported to the posterior lobe of the pituitary by protoplasmic flow in axons of the supraoptico-hypophyseal tracts was first enunciated by Sharrer a number of years ago. The material formed in the neurons, i.e. the neurosecretory material, is thought to be a protein of molecular weight of 30,000 to which is bound one molecule of antidiuretic hormone and one of oxytocin. The pituicytes of the posterior lobe of the pituitary, once considered to be the actual secreting cells, are now believed to play some role in the release of active hormone fragments from the neurosecretory protein in the nerve terminations.

*Nature of the Antidiuretic Hormone.* DuVigneaud has determined the structure and synthesized two antidiuretic hormones derived respectively from beef and hog pituitaries. Arginine-vasopressin is derived from beef pituitaries and probably occurs in the glands of man, monkey, dog, rat, sheep and camel. Lysine-vasopressin is derived from hog pituitaries.



Arginine-Vasopressin (Beef)



Lysine-Vasopressin (Hog)

Commercial pitressin is a mixture of the two. The hormones can be considered as octapeptides consisting of a 5-membered ring made up of tyrosine, phenyl alanine, glutamine, asparagine and cystine and a 3-membered side chain of proline, either arginine or lysine, and glycinamide. In the structures shown above, two molecules of cysteine are joined in disulfide linkage to form a closed ring; i.e., a ring containing one molecule of cystine.

The term vasopressin is strictly a misnomer and derives from the common method of assay of commercial preparations in terms of their potency in elevating blood pressure of the experimental animal. The hormone is many orders of magnitude more active in its antidiuretic action on the kidney than as a pressor agent. It is probably never secreted in amounts necessary to raise blood pressure significantly.

*Role of Antidiuretic Hormone in the Control of Osmolality.* If an individual ingests rapidly two liters of water and every

U/P ratio is a more sensitive indicator of water conservation than is urine flow when flow is very low. It is apparent that the infusion of 7.5 milliunits of pitressin per hr. decreased urine flow of a normal man from 16 ml. per min. to 1.4 ml. per min. and increased creatinine U/P ratio from 7 to 80. Increasing the rate of pitression infusion first to 18 and then to 50 milliunits per hr. further depressed urine flow from 1.4 to 0.6 ml. per min. and increased creatinine U/P ratio from 80 to 180. The renal response to the infusion of 50 milliunits of pitressin per hr. was the greatest that could be attained, and is comparable to that observed in moderate dehydration. It is, therefore, reasonable to assume that the dehydrated subject produces antidiuretic hormone at essentially this rate. According to Lauson, normal man regulates urine flow over the physiological range by variably secreting antidiuretic hormone at rates of 0.1 to 0.8 milliunits per hr. per Kg. body weight. Shannon and Verney have found comparable rates of secretion in the dog.

*Site and Cellular Mechanism of Action of Antidiuretic Hormone.* In Chapter IV it was pointed out that antidiuretic hormone controls the permeability of the distal tubules and collecting ducts to water. Under conditions of maximum hydration, i.e. in the absence of circulating ADH, these structures are impermeable to water. Continued reabsorption of sodium results in the excretion of a large volume (15 to 20 ml. per min.) of hypotonic urine. Under conditions of hydropenia, i.e., in the presence of a high titre of circulating ADH, both distal tubules and collecting ducts are freely permeable to water. Hypotonic fluid, entering the distal tubules from the loops of Henle, loses water to and equilibrates osmotically with blood in the cortical capillaries. Continued reabsorption of sodium, coupled with free transfer of water, reduces volume at the end of the distal tubules to 3 to 6 ml. per min. This fluid is isosmotic with systemic blood. In the collecting ducts water is lost to the hypertonic medullary and papillary tissue. The urine is reduced in volume and becomes as hypertonic to systemic blood as the tissue at the tip of the papilla.

Koefoed-Johnsen and Ussing observed that antidiuretic hormone greatly increases the permeability of isolated frog skin to water. Their analysis led them to believe that the skin is penetrated

effective in defending the body against dilution than against dehydration. Ultimately thirst must drive the individual to replace water deficits; the kidney cannot replace them.

*Rate of Antidiuretic Hormone Secretion.* The condition of maximum sustained hydration described above is a favorable one in which to assess experimentally the probable range of normal rate of secretion of antidiuretic hormone. Verney and Shannon have studied this problem in the dog, Lauson has done so in man. If, when urine flow has stabilized at the high rate of 15 to 20 ml.

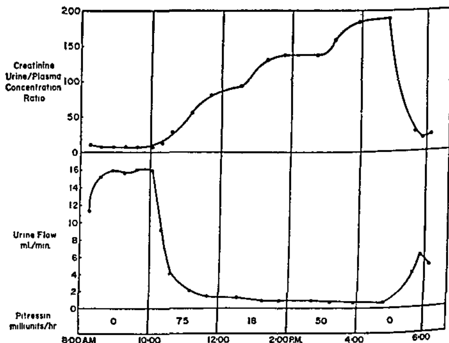



Fig 17. Effects of Pitressin in increasing amounts on creatinine urine plasma concentration ratio and urine flow of normal man. (Redrawn from data of H.D Lauson *Am. J. Med*, 11·135, 1951.)

per min., antidiuretic hormone is infused intravenously in low dosage, urine flow falls and the concentration of various urinary constituents increases. The degree to which creatinine is concentrated in the urine relative to the plasma, i.e, the creatinine U/P ratio, is a useful measure of the avidity with which the kidneys conserve water. As is apparent in Figure 17, the creatinine

supraoptic and paraventricular nuclei or in the adjacent hypothalamic tegmentum; a neurosecretory system which releases the hormone pitressin in amounts related to the need for conserving water; and a renal effector mechanism which responds to the titre of circulating hormone by varying urine flow. In the hydrated individual, pitressin secretion is inhibited, that which circulates is metabolized, and the distal tubules and collecting ducts become impermeable to water. A large volume of dilute urine is excreted. In the dehydrated individual, pitressin secretion is stimulated, the distal tubules and collecting ducts become permeable to water, and water reabsorption is enhanced. A small volume of concentrated urine is formed.

## REFERENCES

1. Epstein, F. H.: Renal excretion of sodium and the concept of a volume receptor. *Yale J. Biol. & Med., 29*:282, 1956.
2. Farrell, G.: Regulation of aldosterone secretion. *Physiol. Rev., 38*:709, 1958.
3. Koefoed-Johnsen, V. and Ussing, H. H.: The contributions of diffusion and flow to the passage of $D_2O$ through living membranes Effect of neurohypophyseal hormone on isolated anuran skin. *Acta physiol. scandinav., 28*:60, 1953.
4. Lauson, H. D.: The problem of estimating the rate of secretion of antidiuretic hormone in man. *Am. J. Med., 11*:135, 1951.
5. Rauschkolb, E. W. and Farrell, G. L.: Evidence for diencephalic regulation of aldosterone secretion. *Endocrinology, 59*:526, 1956.
6. Smith, H. W.: Salt and water volume receptors. An exercise in physiologic apologetics. *Am. J. Med., 23*:623, 1957.
7. Thorn, N. A.: Mammalian antidiuretic hormone. *Physiol. Rev., 38*: 169, 1958.
8. Verney, E. B.: Water diuresis. *Irish J. Med. Sc., 345*:377, 1954.

by minute channels or pores, through which water flows when solutions of different osmolal concentrations are in contact with the two surfaces. In the absence of antidiuretic hormone these pores are small and the transfer of water is slow. In the presence of antidiuretic hormone the pores are greatly dilated and the osmotic transfer of water is rapid. Sawyer, Berliner and Gottschalk have suggested that antidiuretic hormone may exert its entire action on the renal tubule in a similar fashion, namely by dilating aqueous channels in cells of the distal tubules and collecting ducts, thus permitting osmotic equilibration of tubular contents and peritubular fluid.

## SUMMARY

The volume of the extracellular fluid compartment is determined by its sodium content, the osmolal concentration, by its water content relative to sodium. Although volume and osmolality are interrelated, they are separately regulated by mechanisms which are at least semi-independent.

The volume regulatory mechanism consists of receptors, which sense either volume or some derivative of volume such as pressure or flow; an integrative center, probably located in the hypothalamus; and a neurohumoral effector mechanism which controls the renal excretion of sodium. In health, sodium excretion precisely balances intake; accordingly, the sodium content and the volume of the extracellular compartment are held constant within narrow ranges of normal. The rate of excretion of sodium is dependent both on the rate at which sodium is delivered into the renal tubules in the glomerular filtrate and on the capacity of the tubules to reabsorb sodium from the urine. Control of excretion is effected by vasomotor nerves and by humoral agents which regulate glomerular filtration rate and by the secretion of aldosterone which regulates tubular reabsorptive capacity. Other less adequately defined mechanisms may also affect the excretion of sodium.

The mechanism regulating osmotic pressure consists of receptors, located within the zone of distribution of the internal carotid arteries and sensitive to changes in osmolality of the arterial blood of the order of $\pm$ 2 per cent; an integrative center, either in the

shown on the left, the extracellular fluid volume of a normal 70 Kg. man is roughly 14 liters. Enough salt and water are delivered into this volume by way of the gastrointestinal tract to cause it to expand at a rate of one-half to one or more liters per day. However, the normal individual is able to adjust the output of salt and water to the intake and thereby to maintain constancy of extracellular fluid volume. The edematous patient, in contrast, exhibits a relative incapacity to excrete salt and water and as a consequence, extracellular fluid volume expands. If, as shown in the diagram on the right, intake is markedly reduced, it may be brought into line with excretory capacity, and fluid volume may be maintained within normal limits, even though the basic and precipitating disease process persists. In this chapter, present concepts of the factors which cause this relative incapacity to eliminate salt and water will be outlined briefly.

*Glomerulo-tubular Balance.* It is evident from the discussion in the preceding chapters that the maintenance of sodium balance involves the failure to reabsorb a minute fraction of the total filtered load. It is, therefore, difficult to determine in any specific instance whether retention of sodium results from a deficiency in the filtered load or from enhanced tubular reabsorption of that load. One can grossly define the problem of maintenance of salt and water balance, and the nature of the disturbances which lead to accumulation of edema in terms of glomerulo-tubular balance. This concept is illustrated in Figure 19. The normal individual filters a normal amount of salt and water through his glomeruli, reabsorbs a normal amount, and hence excretes a normal amount. He, therefore, exhibits glomerulo-tubular balance. The quantity of salt and water excreted is usually 1 per cent or less of that filtered, accordingly, 99 per cent or more is reabsorbed.

If filtration rate is reduced and if there is no corresponding reduction in tubular reabsorptive activity, excretion decreases as indicated in the middle diagram. Conversely, if filtration rate is normal and if tubular reabsorptive activity is increased, as shown on the right, excretion likewise decreases. The small glomerulus in the middle diagram and the heavier tubule on the right are drawn to represent alterations in function, not morphologic changes. The

*Chapter VI*

# RENAL FACTORS IN EDEMA FORMATION

EDEMA is a clinical sign, a manifestation of diseases of varied origin, not a disease entity in its own right. There are common features in the pathogenesis of edema in condition as varied as congestive heart failure, cirrhosis, nephrosis, nephritis, preeclampsia, protein starvation, and even thrombophlebitis; there are obviously differences as well. In this chapter, the common features will be emphasized. Furthermore, the edema of congestive failure

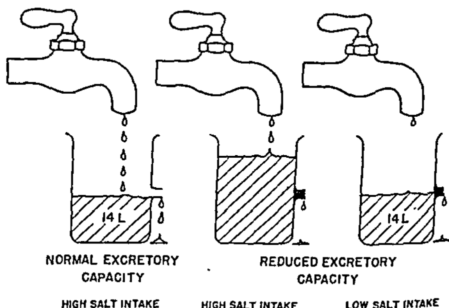



Fig. 18. Salt and water intake and excretory capacity in relation to edema

will receive primary consideration, for the problem is not only the most common but has also been most intensively studied.

*The Role of the Kidneys in the Retention of Salt and Water in Edema* is illustrated in diagrammatic fashion in Figure 18. As

some 35 per cent of the sodium contained in an intravenous load of 500 ml. of isotonic saline in the first hour and about 80 per cent in 8 hours. Tricuspid insufficiency alone reduced salt tolerance considerably, i.e., it reduced excretion during the first hour markedly, and total 8 hour excretion appreciably. These animals exhibited little or no additional evidence of circulatory inadequacy. The combined lesions of pulmonary stenosis and tricuspid insufficiency, bringing out the full blown syndrome of congestive right heart failure, reduced excretion of the sodium load to insignificant proportions, both at 1 and at 8 hours. These results are reminiscent of those of Schroeder, Burch and others who have shown that patients in congestive failure excrete a sodium load less readily than do normal individuals and that tolerance to sodium loading diminishes with increasing signs of congestion.

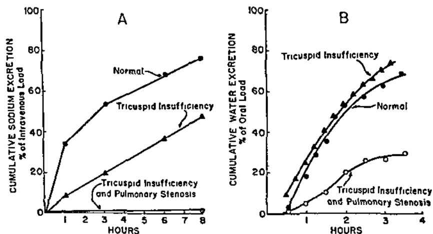



Fig 20. Effects of production of tricuspid insufficiency and tricuspid insufficiency plus pulmonary stenosis (chronic congestive heart failure) in the dog, A, on the excretion of an intravenous load of 500 ml of isotonic saline, B, on the excretion of an oral load of 500 ml of water (From A. C. Barger· *Metabolism,* 5:480, 1956.)

Barger has also demonstrated that in severe experimental right heart failure water diuresis is blunted. However, water excretion is by no means depressed to the same extent as sodium excretion. Total cumulative water excretion in these same experimental animals is shown in Figure 20B. Tricuspid insufficiency alone pro-

two diagrams to the right represent the two possible causes of glomerulo-tubular imbalance characterized as tubular preponderance. Reduced excretion, independent of the cause, leads to expansion of extracellular stores of salt and water and to the accumulation of edema.

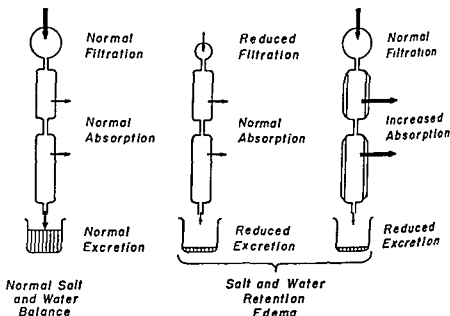



Fig. 19. The concept of glomerulo-tubular balance in relation to regulation of salt and water reserves of the body.

*Limitation of Excretory Capacity for Salt and Water in Experimental Congestive Failure.* Barger has recently shown that chronic congestive right heart failure may be induced in the dog by the combined lesions of a 50 per cent stenosis of the pulmonary artery and avulsion of the leaflets of the tricuspid valve. Such animals exhibit elevated right atrial pressure, reduced cardiac output, reduced exercise tolerance, ascites and edema.

The data shown in Figure 20A illustrate the progressive reduction in the capacity of an animal to excrete a standard sodium load when first, tricuspid insufficiency and later, pulmonary stenosis are induced. In control experiments, normal dogs excreted

*Metabolic* factor. Among the *Hormones*, those of the adrenal cortex, the antidiuretic hormone, and both epinephrine and norepinephrine have been implicated as playing causal roles.

Present evidence indicates that two factors, namely reduction in glomerular filtration rate and increased secretion of the adrenal salt retaining steroid, aldosterone, are the primary causes of the glomerulo-tubular imbalance which results in salt retention in edema. Renal anoxia, renal venous congestion, and stimulation of sodium reabsorption by renal nerves and/or by adrenal medullary amines play minor roles, if indeed they are of any significance in the pathogenesis of edema. In the interest of brevity and to avoid an overly contentious discussion, only the major factors will be considered in any detail. However, the role of antidiuretic hormone must be treated briefly.

*Antidiuretic Hormone.* The oft repeated observations that edematous patients exhibit delayed and depressed water diuresis and that plasma sodium and osmolality are frequently subnormal in severe congestive failure, in cirrhosis with ascites, and in nephrosis suggest excessive antidiuretic hormone activity. Some have gone so far as to state that water retention in edema is primary and that salt retention is secondary to body dilution. A more reasonable view is that excessive antidiuretic hormone activity may be a contributory cause of the hyponatremia of severely ill patients, but that sodium retention, not water retention is the basic abnormality in edema.

Goodman and Gilman were the first to demonstrate the presence of an antidiuretic substance in the urine and to show that its rate of excretion is related to the need for water conservation. Subsequently, excessive rates of urinary excretion of antidiuretic substances were noted in patients with cirrhosis and ascites by Ralli, Lloyd, and Sims, with congestive failure by Bercu and Dochios; with eclampsia by Teel and Ham, with hypertension by Grollman and with Bright's disease by Robinson. In general it has been obesrved that rate of excretion of antidiuretic substances is high during the phase of active accumulation of edema and low during the diuretic phase. In cirrhosis, antidiuresis has been variously ascribed to reduced destruction of hormone by damaged liver

duced no significant alteration in water diuresis. Excretion of water was normal, in fact slightly greater, in the dogs with the single lesion. On the other hand, the combined lesions of tricuspid insufficiency and pulmonary stenosis reduced the diuretic response to a standard water load by roughly one half. Again Barger's findings in experimental right heart failure are reminiscent of those of Schemm, Proger and others who have shown that patients in congestive failure excrete a water load somewhat less readily than do normal subjects.

## FACTORS LIMITING SALT AND WATER TOLERANCE

In Table V are listed a number of factors to which this marked reduction in sodium and moderate reduction in water tolerance have been ascribed. These factors include the *Hemodynamic* ones of reduced renal blood flow, reduced glomerular filtration rate, and elevated venous pressure. Since changes in renal blood flow and filtration rate may well be mediated in part through the sympathetic nervous system, the second term, *Neural Factor* is used in a restricted sense to mean a direct control of the reabsorptive activities of the renal tubules by nerve impulses. Relative anoxia, as a consequence of renal ischemia, was long considered a dominant

TABLE V

RENAL FACTORS IN THE RETENTION OF SODIUM AND WATER IN EDEMA

I. *Hemodynamic* (neurally and hormonally mediated)
   1. Reduction in Renal Blood Flow and Glomerular Filtration Rate
   2. Elevation of Renal Venous Pressure

II. *Neural* (independent of hemodynamic)
   1. Nervous Stimulation of Renal Tubular Reabsorption of Sodium

III. *Metabolic*
   1. Anoxia

IV. *Hormonal*
   1. Aldosterone Excess
   2. Pitressin Excess
   3. Epinephrine and Nor-Epinephrine Excess

and osmo-regulatory mechanisms are not entirely independent. Peters maintains that although the hypothalamic-hypophyseal antidiuretic hormone mechanism is primarly responsive to changes in osmolality, it also responds to absolute or relative inadequacy of blood volume. The severely ill patient, or the patient subjected to massive paracentesis or diuresis may sacrifice normal osmolality to expand volume. Whether such a patient secretes excessive quantities of antidiuretic hormone in an absolute sense is uncertain. Relative to needs for maintenance of normal osmotic relationships, the secretion of any hormone is excessive.

*Reduction in Renal Blood Flow and Glomerular Filtration Rate.*That alterations in renal hemodynamics might play a significant role in the pathogenesis of edema in congestive failure was first clearly expressed more than a decade ago by Warren, Stead, Merrll, Mokotoff and others. They observed that renal blood flow is markedly reduced and glomerular filtration rate moderately reduced in edematous patients in severe congestive failure. Most of their patients with filtration rates less than 70 ml. per min. were frankly edematous. Those with greater filtration rates, especially those with rates approaching the normal range of 120 to 140 ml. per min. were not. They concluded that reduction in filtration rate without equivalent reduction in tubular reabsorptive activity leads to retention of salt and water and to the formation of edema.

Moderate exertion, in fact even the assumption of the erect posture, leads to an appreciable decline in renal blood flow and glomerular filtration rate in the normal subject. Accordmg to Merrill and Cargill, a number of patients who are compensated and edema-free when acuvity is restricted, suffer a marked fall in glomerular filtration rate to or below the presumed cntical level of 70 ml. per min. and expand extracellular fluid reserves when activity is greater and more prolonged. Renal hemodynamic changes include constriction of both afferent and efferent glomerular arterioles. Constriction of afferent arterioles lowers filtration pressure and reduces filtration rate; constriction of both afferent and efferent arterioles reduces blood flow to a more marked degree than filtration rate. These hemodynamic changes have been ascribed on the one hand to enhanced vasoconstrictor nerve

tissue and to enhanced secretion of hormone due to the presence in serum of high concentrations of ferritin. In a few instances the concentration of antidiuretic substances in the serum of edematous patients has been observed to be greater than normal.

This seemingly convincing evidence has been called into question by Van Dyke who points out the lack of specificity of the assay methods employed. For the most part, it has not been proved that the antidiuretic materials are hormonal in nature and derived from the neurohypophysis. It is thoroughly possible that the materials extracted from the urine of edematous patients cause the liberation of hormone from the neurohypophysis of the assay animal and are not in themselves antidiuretic.

Recently Laragh has shown that ascites can be produced in the dog with diabetes insipidus by constriction of the inferior vena cava above the liver. These animals continue to show marked polydipsia and polyuria during accumulation of ascitic fluid, the rate of accumulation being proportional to salt intake, not water intake. Apparently the accumulation of ascites is in no wise dependent on the presence of an intact neurohypophysis, much less on the presence of an overactive one. A great deal of evidence points to the fact that alcohol produces its familiar diuretic response by inhibiting the release of antidiuretic hormone from the pituitary gland. Lamdin, however, has found that even repeated doses of alcohol to patients with congestive failure, cirrhosis, and nephrosis, do not restore the diuretic response to normal; in fact do not affect it at all. The implication is that excessive circulating antidiuretic hormone activity plays no role in the reduced diuretic response of edematous patients.

It is the authors personal view that antidiuretic hormone basically play the same role in the edematous patient that it plays in the normal individual. It is secreted in amounts sufficient to control water excretion and to maintain osmolality of the body fluids within normal limits. Its presence in excess is by no means necessary for accumulation of edema and ascites. Delayed and depressed diuresis may be a consequence of glomerulo-tubular imbalance rather than of excessive antidiuretic hormone activity. However, it was pointed out in Chapter V that volume regulatory

the same extent in response to a reduction in filtration rate. Decreased excretion resulted from a relative increase in tubular reabsorption of salt and water. The response was obviously independent of renal nerves, the posterior lobe of the pituitary, and the adrenal glands; it appeared to be primarily related to the reduction

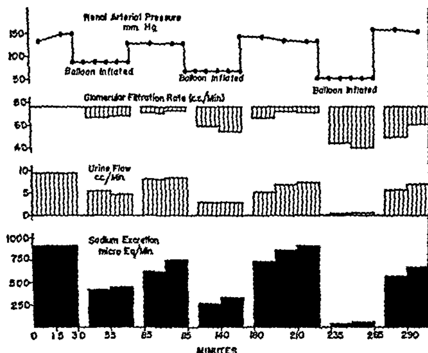



Fig 21. The effects of controlled reduction of renal arterial pressure in the dog on glomerular filtration rate, urine flow and sodium excretion. (Drawn from data of D.D. Thompson and R.F. Pitts. *Am. J. Physiol.*, 168:490, 1952.)

in filtration rate. At a filtration rate of 50 per cent of normal, roughly comparable to Merrill's value of 70 ml. per min. in man, reabsorption of sodium and water was essentially complete.

These experiments of Thompson demonstrate that an acute reduction in filtration rate, similar to that which occurs on assuming the erect posture and on mild to moderate exertion in the patient with reduced cardiac reserve leads to an abrupt reduction in the excretion of salt and water. What is the significance of

activity, on the other, to the liberation of renin by the kidney and *the formation of the vasoconstrictor material, angiotonin,* in the bloodstream.

Kattus, Briggs and others, observing recovery of compensation and loss of edema without increase in renal blood flow or glomerular filtration rate, have minimized the significance of renal hemodynamic factors in the pathogenesis of edema in congestive failure, or have even denied their existence. However, the fact that *reduction of filtration rate does indeed lead to more complete* reabsorption of sodium and water by the renal tubules has been amply demonstrated in experimental animals by Selkurt, Duggan, Mueller, Thompson and others.

In the studies of Thompson, a Dotter-Lukas balloon catheter was introduced into the femoral artery of a dog and positioned in the aorta immediately rostral to the origins of the renal arteries. *By inflating the balloon, the renal arterial pressure could be* reduced to, and stabilized at any desired value. Figure 21 summarizes the effect of controlled inflation of the balloon on renal arterial pressure, glomerular filtration rate, urine flow and sodium excretion in a respresentative experiment on an anesthetized dog. Prior to and during this experiment the animal was infused with isotonic saline at a rate of 10 ml. per minute. As a consequence of *saline loading, high rates of urine flow and sodium excretion were* observed in the initial two control periods. Reduction of renal arterial pressure from 150 to 90 mm. Hg. reduced filtration rate to a minor degree, namely from 77 to 70 ml. per min. However, this 10 per cent reduction in filtration rate was associated with a 50 per cent reduction in sodium excretion. Thus sodium excretion decreased from 900 to 450 $\mu$Eq. per min. Inflation of the balloon a second time, reducing renal arterial pressure to 70 mm. Hg, caused a further decline in filtration rate and sodium excretion. Inflation a third time reduced filtration rate roughly by half, that is, to 40 ml. per min. Excretion of sodium to all intents ceased.

Thompson *studied the renal response to a reduction in renal* perfusion pressure in normal, in sympathectomized, in adrenalectomized, and in diabetes insipidus dogs. Under all experimental conditions, sodium excretion and urine flow decreased to essentially

the same extent in response to a reduction in filtration rate. Decreased excretion resulted from a relative increase in tubular reabsorption of salt and water. The response was obviously independent of renal nerves, the posterior lobe of the pituitary, and the adrenal glands; it appeared to be primarily related to the reduction

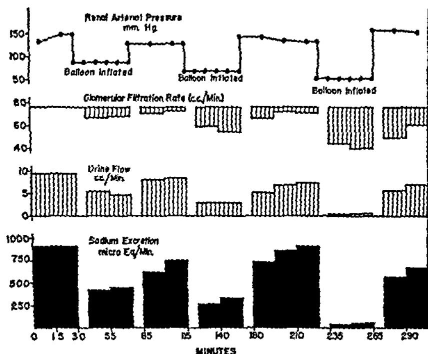



Fig. 21 The effects of controlled reduction of renal arterial pressure in the dog on glomerular filtration rate, urine flow and sodium excretion. (Drawn from data of D.D. Thompson and R.F. Pitts: *Am J Physiol.*, *168*:490, 1952.)

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These experiments of Thompson demonstrate that an acute reduction in filtration rate, similar to that which occurs on assuming the erect posture and on mild to moderate exertion in the patient with reduced cardiac reserve leads to an abrupt reduction in the excretion of salt and water. What is the significance of

reduction in filtration rate in the long term regulation of fluid and electrolyte balance?

Mueller has shown that constriction of one renal artery in a dog with ureters separately exteriorized results in a reduction in glomerular filtration rate and in sodium excretion on the constricted side which persists for weeks. However, no change in either volume or composition of the body fluids occurs, for the normally functioning kidney maintains proper balance. When the normal kidney is removed, salt reabsorption is reduced and salt exretion increased on the side of renal artery constriction, presumably in response to a change in volume or composition of the extracellular fluid. Glomerulo-tubular balance is re-established by a reduction in tubular reabsorptive activity.

One may interpret the role of reduced filtration rate in the pathogenesis of edema in the light of these findings as follows. An acute reduction in filtration rate leads to salt and water retention and to a slight but significant expansion of extracellular fluid volume. The otherwise normal individual responds within a day or so by decreasing his rate of secretion of salt retaining adrenal steroids. Glomerulotubular balance is restored by reduction of tubular reabsorptive activity. No appreciable edema fluid accumulates. Certain patients with reduced cardiac reserve, under the stress of exertion, of infection or of progress of their disease, suffer an equivalent reduction of filtration rate. They do not reduce their rate of secretion of steroids, at least not to a degree sufficient to re-establish glomerulo-tubular balance. Accordingly, edema fluid accumulates.

*Increased Secretion of Adrenal Cortical Steroids.* Following the demonstration by Loeb and by Harrop of the significance of the adrenal cortex in the renal tubular reabsorption of sodium, there have appeared numerous references to the possible role of increased adrenal cortical activity in the pathogenesis of edema in congestive failure, cirrhosis, pre-eclampsia, nephrosis and nephritis. This view first gained strength from the observation that Addisonian patients, overtreated with desoxycorticosterone became edematous. More recently it has found support in the appearance of Cushing's syndrome in patients treated with large doses of

cortisone or ACTH. Several additional lines of evidence have implicated an adrenal cortical factor. In edematous patients, whatever the underlying disease, sweat and salivary sodium concentrations and fecal sodium excretion are low. Such effects can be induced in otherwise normal individuals by the administration of salt retaining steroids. According to Luetscher, Bartter and others, the urinary excretion of the salt retaining factor, aldosterone, is

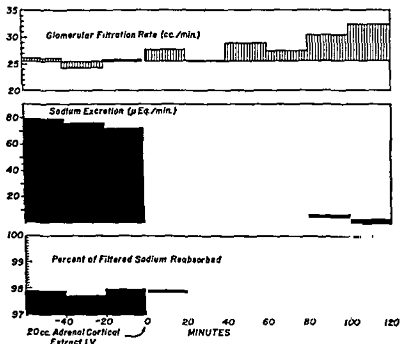



Fig 22 The effects of intravenous administration of whole adrenal cortical extract on glomerular filtration rate and reabsorption and excretion of sodium in the adrenalectomized dog (Drawn from data of J.C. Roemmelt, O W. Sartorius, and R.F. Pitts· *Am. J. Physiol.*, 159 124, 1949)

increased in congestive failure, cirrhosis, nephrosis and nephritis. Finally bilateral adrenalectomy may cause diuresis and loss of edema and ascitic fluid in dogs with experimental ascites, and in hypertensive and cirrhotic patients

One must remember that adrenal hormones affect the renal tubular reabsorption of a very small, though highly significant

fraction of the filtered sodium. The experiment on an adrenalectomized dog, summarized in Figure 22, was performed by Roemmelt some years past when cortisone was still unobtainable and aldosterone was an unknown component of the amorphous fraction. Replacement hormone therapy was withdrawn 4 days before the experiment. For 3 days the dog was allowed 0.6 per cent saline ad lib, the last day, only tap water. During the 3 control periods, 70 to 80 $\mu$Eq. of sodium were excreted per min. This represented a failure to reabsorb only 2 per cent of the filtered sodium, for as shown at the bottom of the graph, reabsorption was 98 per cent complete. However, this minor defect in sodium reabsorption is highly significant; were it maintained over a period of days, extracellular reserves of salt and water would be seriously, possibly fatally depleted. At the break in the graph, 20 ml. of whole adrenal cortical extract was given intravenously. After a lag phase of 40 minutes, sodium reabsorption increased to become 99.9 per cent complete and urinary sodium excretion decreased essentially to zero.

Were such low rates of excretion of sodium to be maintained in the face of luxus intake, body sodium stores would of course progressively expand. To a certain extent this must occur in the Addisonian patient overtreated with desoxycorticosterone and in the patient with normal adrenal function treated with large doses of cortisone or ACTH. Why does it not occur in patients with primary aldosteronism, in some of whom higher rates of urinary excretion of salt retaining hormone are observed than in patients in congestive failure? Why cannot one make a normal dog or a normal man edematous with large doses of desoxycorticosterone?

The answer in part may be that the normal individual exposed to high salt retaining hormone activity increases filtration rate and filtered sodium load sufficiently to offset increased tubular reabsorption. The patient in congestive failure does not. We have thus come full circle in our argument. The patient in congestive failure with low filtration rate does not reduce salt retaining hormone output sufficiently to compensate for his reduced filtered load of sodium. Similarly the patient in congestive failure with excessively high salt retaining hormone output does not increase

the filtered sodium load sufficiently to compensate for increased reabsorptive activity. In either instance, glomerulo-tubular imbalance exists and tubular preponderance accounts for the progressively expanding sodium stores. It is not surprising, therefore, that certain patients are edematous despite the fact that their filtration rates are normal. Nor should it be surprising to find that others are edematous despite normal rates of excretion of salt retaining hormone. When considering glomerulo-tubular balance, normal is strictly a relative term.

In a study of Bartter summarized in Figure 23A, a normal subject was depleted of sodium by maintenance on a low salt intake and by oral cation exchange resin. Urinary excretion of aldosterone was high, roughly 50 micrograms per day,[17] and urinary and fecal excretion of sodium were negligible over the first 6 days of the study. On days 9 through 14, 2 liters of isotonic saline were given intravenously each day. Urinary aldosterone excretion decreased promptly and both urinary and fecal sodium excretion increased. Reinstitution of low sodium intake led to an abrupt increase in excretion of aldosterone and to a reduction in urinary and fecal sodium losses. The normal individual obviously responds promptly to variations in salt content of the diet by altering urinary output of aldosterone. The edematous patient responds in a qualitatively similar fashion but more sluggishly and the hormonal regulatory mechanism seems set at a higher level of activity.

In a similar study on a patient in congestive failure summarized in Figure 23B, gradual weight loss and decline of venous pressure resulted from a low sodium diet and oral resin therapy. Even though the patient was frankly edematous at the start of the period of observation, his rate of excretion of aldosterone was appreciable, amounting to 10 μgm. per day. Had this individual not been suffering from circulatory inadequacy, it is logical to assume that a

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[17] It is tacitly assumed that urinary excretion of aldosterone reflects rate of secretion of hormone by [illegible]
in the body fluids [illegible]
mented that it can [illegible]
salt intake excretes f[illegible]
he may excrete from 10 to 50 μgm. per day. In contrast the patient in congestive failure excretes from 10 to 60 μgm. per day.

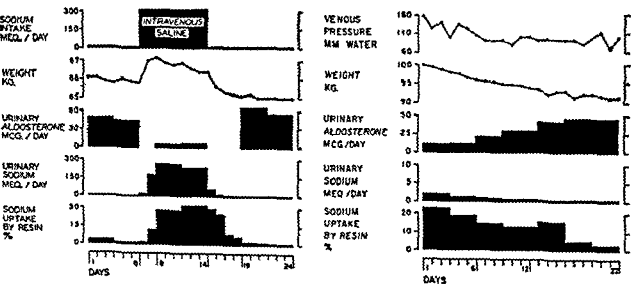



Fig. 23. Effects of sodium deprivation and sodium loading on urinary and fecal excretion of sodium, on body weight, and on urinary excretion of aldosterone. A. Study on a normal man B. Study on a patient in congestive failure. (From L.E. Duncan, Jr., G.W. Liddle, and F.C. Bartter: *J Clin. Invest.*, *35*:1299, 1956.)

much less significant expansion of extracellular fluid volume would have caused his rate of excretion of hormone to drop essentially to zero. With progressive depletion of body sodium stores and an approach to a non-edematous state, urinary aldosterone excretion rose to high levels. Obviously the set of the mechanism controlling secretion of aldosterone is different in the patient with reduced cardiac reserve Is this mechanism sensitive to the total body sodium store, to sodium concentration, to extracellular volume or to some derivative of extracellular volume such as intravascular volume or pressure?

According to Bartter, Mach, Muller, Vesin and others, the mechanism regulating aldosterone secretion is sensitive to extracellular volume or to some derivative of volume. As shown in Table 6, urinary excretion of aldosterone is decreased by three procedures, each of which increases extracellular volume, namely the administration of water and pitressin, the administration of 3 per cent saline and the administration of isotonic saline. On the other hand, aldosterone excretion is increased by two procedures which reduce extracellular volume, namely water privation and mercurial diuresis. There seems to be no correlation of hormone excretion with intracellular volume, serum sodium and either intracellular or extracellular osmolality

One must temper ones enthusiasm for this thesis with the reservation that rate of urinary excretion may not be an infallable indication of rate of glandular secretion of aldosterone. Davis has shown in the dog, that less than 1 per cent of the glandular output is excreted in the urine. The possibility that any given factor may alter urinary excretion by affecting metabolism of hormone or renal tubular reabsorption of hormone rather than by affecting rate of glandular secretion must always be considered. Reduced hepatic destruction of hormone by the patient with cirrhosis or with an engorged congested liver is a likely but unproven possibility.

It is by no means accepted by all that the volume of extracellular fluid is the sole or even the major factor controlling aldosterone secretion. Deane, Singer *et al.*, and Laragh consider that the body store of potassium exerts a major controlling influence. a high

## TABLE VI

REGULATION OF ALDOSTERONE SECRETION

| | *Extracellular Volume* | *Intracellular Volume* | *Extracellular Osmolality* | *Intracellular Osmolality* | *Serum Sodium* |
|---|---|---|---|---|---|
| *Factors Decreasing the Secretion of Aldosterone* | | | | | |
| Hydration plus pitressin | + | + | – | – | – |
| Infusion 3% NaCl | + | – | + | + | + |
| Infusion 0.9% NaCl | + | O | O | O | O |
| *Factors Increasing the Secretion of Aldosterone* | | | | | |
| Thirsting | – | – | + | + | + |
| Mercurial diuresis | – | O | O | O | O |

potassium store increasing, a low potassium store decreasing aldosterone secretion. Others have suggested that a receptor mechanism sensitive to serum sodium concentration regulates aldosterone output. The evidence for this view is not impressive. Perhaps as Farrell suggests, many factors can affect aldosterone secretion. Of these, the factor of volume of extracellular fluid is the one most pertinent to our discussion.

*Role of Colloid Osmotic Force in Fluid Retention.* Vander *et al* have recently postulated that retention of fluid and electrolyte in congestive heart failure is related to a greater reduction in renal blood flow than in glomerular filtration rate, i.e., to the increase in filtration fraction commonly observed in this condition. They point out that the filtration of a greater than normal fraction of the plasma perfusing the kidney results in an increase in the colloid osmotic pressure of the peritubular blood. They postulate that the oncotic force exerted by plasma proteins in peritubular capillaries is normally responsible for the reabsorption of fluid in the proximal tubule and that an increase in this force in congestive failure accounts for the over-reabsorption of fluid which results in edema.

To maintain proper perspective one must consider the quantitative aspects of their thesis. The oncotic force normally exerted by the plasma proteins in the peritubular capillaries is roughly 31 mm. Hg, slightly less than the equivalent of 2.0 mOsm. per liter concentration difference. Were filtration fraction to increase from the normal value of 0.2 to peak value of 0.5 in congestive failure, the colloid osmotic force would be increased to 50 mm. Hg, roughly the equivalent of 3.0 mOsm. per liter concentration difference.

Were any component of the tubular urine to be restricted in its movement relative to water to such an extent that its concentration increased by 2.0 mOsm. per liter in the normal or by 3.0 mOsm. per liter in the patient with congestive failure, all proximal transport would cease. One must remember that the total osmotic pressure of the proximal fluid is 5100 mm. Hg, equivalent to an osmolal concentration of 300 mOsm. per liter. It is difficult to believe that the tubular epithelium is so completely permeable to solutes as to permit passive migration of all in proportion to water

without restriction. Were the colloid osmotic force the only one available to cause reabsorption of fluid, excretory products could be concentrated to a negligible degree in the proximal segment.

Let us reverse the argument. As Bayliss has pointed out (see page 61), the osmotic force created by the *active reabsorption* of glucose from filtrate containing 100 mg. per cent is some 2.5 times the colloid osmotic force. In the mild or reasonably well controlled diabetic excreting no sugar but with blood glucose elevated to 200 mg. per cent, the osmotic force created by active reabsorption of sugar is some 5.0 times the colloid osmotic force. Were the tubule as freely permeable to salt and water as postulated by Vander *et al.*, mild diabetics would be considerably more edematous than patients in congestive failure.

In Chapter IV the author has developed the thesis that the reabsorption of sodium is active and provides the motive force for the reabsorption of the bulk of the filtered water in the proximal tubule. The active transport of any so-called threshold solute, such as glucose or amino acid, contributes to this force. Even the colloid osmotic force of plasma proteins in peritubular capillaries contributes, but the contribution is minor. It certainly cannot account for the reabsorption of four-fifths or more of the filtrate under normal conditions nor for *fluid and electrolyte retention in congestive failure.*

*Compensatory Elements in the Fluid Retention of Edema.* Why are renal salt retaining mechanisms so entrained in a variety of diseases as to lead to the formation of edema and ascites? Does expansion of extracellular fluid volume serve a useful purpose for the patient, is it an expression of the operation of a normal homeostatic mechanism carried to extremes in disease and therefore of limited use or actually deleterious for the patient, or is it strictly a pathologic result of disease serving no useful purpose whatever? No exact answer is possible. In our present state of knowledge, the question must be answered more on the basis of philosophy than of fact. However, Peters, Landis, Borst, and a number of others are more or less in agreement with the second of the possibilities outlined above; i.e., fluid retention is basically compensatory; carried to extremes of edema, it is deleterious.

As was pointed out in Chapter V, three simple procedures result in a prompt reduction in excretion of salt and water by normal subjects: quiet, erect standing, the application of venous tourniquets to the thighs, or the removal of a pint or so of blood from a peripheral vein. These same procedures induce a sensation of thirst. Common to all, are reduction in venous return, diminished distension of central and cephalic venous channels, and reduced cardiac output. Obviously, the circulatory status in each instance would be improved by increasing circulating blood volume. Presumably the volume receptor mechanism is triggered in some manner and through neural and humoral mechanisms, filtration rate is reduced, tubular reabsorption of salt and water is increased, and fluid intake is stimulated.

It is reasonable to assume that in diseases characterized by edema, circulating blood volume is less than optimum. This does not necessarily mean less than normal per Kg. of body weight. A patient with reduced cardiac reserve or one with cirrhosis, in whom blood is trapped in the portal system, might well have an increased total blood volume, yet an effective volume less than optimum. A patient with nephrosis or with protein undernutrition might well have a volume less than normal in an absolute sense due to failure of oncotic mechanisms to hold fluid in the vascular compartment The volume receptor mechanism, whatever and wherever it is, is triggered. Filtration rate decreases; adrenal cortical secretory mechanisms are activated, salt retention occurs. Thirst results and fluid intake increases. Slight hypo-osmolality develops, the more severe the disease process, the greater the demands for blood volume expansion and the more marked the hypo-osmolality. At best only one third of the retained salt serves a useful purpose in expanding blood volume, for plasma volume makes up a third or less of total extracellular fluid volume, two thirds or more of the salt and fluid is distributed in the tissue interstices as edema. The significant point is the following. Retention of salt and water in the edema of congestive failure and in the ascites of cirrhosis, no less than that which follows quiet standing, hemorrhage or the application of tourniquets to the legs may be basically compensatory insofar as it leads to expansion of blood volume. Insofar as it

abnormally expands interstitial fluid volume as edema, it serves no uesful purpose and is deleterious.

## SUMMARY

At least two factors play dominant causal roles in retention of salt and water in edema. First, renal vasoconstriction, mediated by sympathetic nerve impulses or by renin release, causes reduction in renal blood flow and in rate of glamerular filtration. Reduction in filtered load, slowed transit and prolonged contact of the fluid with the tubular epithelium leads to over-reabsorption of salt and water. Second, an absolute or a relative increase in the secretion of salt retaining adrenal steroids stimulates tubular reabsorption and leads to retention of salt and water.

These two alterations in renal function are synergistic causes of the glomerulotubular imbalance which underlies the accumulation of edema. One cannot point to hypersecretion of aldosterone as the sole cause of fluid retention in a given patient, even though filtration rate is within normal limits. Were the individual otherwise normal, a compensatory increase in filtration rate would re-establish glomerulo-tubular balance despite enhanced tubular reabsorptive activity. Similarly one cannot point to a reduction of filtration rate, even though it falls to less than 50 per cent of normal as the sole cause of long term fluid retention in congestive failure. In the absence of circulatory insufficiency, the rate of secretion of salt retaining adrenal steroids would be reduced to a level sufficient to re-establish glomerulo-tubular balance.

## REFERENCES

1. Barger, A. C.: The pathogenesis of sodium retention in congestive heart failure. *Metabolism, 5:480, 1956.*
2. Borst, J. G. G.: The characteristic renal excretion patterns associated with excessive or inadequate circulation. *Ciba Foundation Symposium on The Kidney*. London, J. & A. Churchill Ltd., 1954, p. 255.
3. Hyatt, R. E. and Smith, J. R.: The mechanism of ascites. A physiologic appraisal. *Am. J. Med., 16:434, 1954.*

J. H., Gilman, A., and Cross, R. J.: *Mechanisms of edema*

formation and principles of management. *Am. J. Med., 21:*423, 1956.
5. PETERS, J. P.: The problem of cardiac edema. *Am. J. Med., 12:*66, 1952.
6. VANDER, A. J., MALVIN, R. L., WILDE, W. S. and SULLIVAN, L. P.: Reexamination of salt and water retention in congestive heart failure. Significance of renal filtration fraction. *Am. J. Med., 25:* 497, 1958.

# Part 2

# Mechanisms of Action and Therapeutic Use of Diuretics

*Chapter VII*

# INTRODUCTION TO DIURETIC THERAPY

IDEALLY, treatment of edema and ascites should be directed toward control of the primary disease and reversal of the pathophysiologic processes which cause expansion of extracellular fluid volume. Within limits, restriction of activity, reduction of weight, and limitation of salt intake can be considered as operating in this fashion in the decompensated cardiac, in some degree by improving the dynamics of the failing heart, in greater degree by reducing the demands upon it. The digitalis glycosides exert their major favorable effects by increasing the work capacity of the damaged myocardium. Steroid therapy in nephrosis and in the nephrotic stage of glomerulonephritis frequently induces remission of, and on occasions dramatically arrests the primary disease. Dietary management in cirrhosis may improve liver function, favorably alter nutrition, increase plasma protein concentration and induce remission of ascites. Diet, rest and salt restriction can in many instances prevent deterioration of the patient with mild pre-eclampsia.

The therapeutic goal of control of the primary disease can rarely be attained in the chronically or severely ill patient, and the physician must resort to diuretics to remove excess fluid from the body. A discussion of the overall management of the patient with edema and ascites is beyond the scope of this monograph; it is limited to a consideration of diuretic therapy and, more specifically, its physiological aspects.

***Definition of Diuretic Agents.*** Diuretics are loosely defiined as agents which increase the volume flow of urine. In this sense, water is the diuretic *par excellence*. However, diuretics are employed in therapeutics to eliminate excess body fluid and to reduce body weight. To achieve these ends by the administration of water, large quantities must be given; many substances are more

effective. A more precise mechanistic definition is that diuretics promote primarily the excretion of sodium and either chloride or bicarbonate, *i.e., those ions which are largely restricted to, and which constitute the major electrolyte components of the extracellular fluid.* Secondarily, water is eliminated in an amount equivalent to the ions excreted, reducing the volume of extracellular fluid and resulting in loss of weight. This definition properly emphasizes the facts that the excretion of ions is primary, that these ions are drawn from extracellular rather than cellular stores, and that increased urine volume and loss of weight are proportional to and the osmotic consequences of loss of ions.

***Properties of the Ideal Diuretic.*** The ideal diuretic should have the following properties. (1) It should be potent, causing adequate diuresis and loss of weight in even the most severely ill patient, irrespective of the nature of his disease. (2) It should cause the excretion of sodium, potassium, chloride, and bicarbonate ions and water in the proportions in which they exist in extracellular fluid, it should cause no electrolyte imbalance due to the preferential excretion of one or another ion. (3) It should be active when used repeatedly; tolerance should not develop. (4) It should be active on oral administration. (5) A single dose should induce a relatively prompt diuresis. (6) It should be non-toxic even when given repeatedly over long periods of time. Needless to say, such a diuretic does not exist. However, there is reason to believe that compounds or combinations of compounds having these properties may eventually be found. Until they are, the practitioner must employ, as best he can, the agents at his disposal.

***Classification of Diuretics.*** A functional classification of diuretics is presented in Table VII. This classification has the virtue that it illustrates the various means by which diuretic agents oppose [illegible] Diuretics, [illegible] ents which [illegible] as or more or less specifically antagonize enhanced tubular reabsorption of those ions. Either action tends to correct glomerulo-tubular imbalance. Filtered load can be increased either by increasing glomerular filtration rate or by increasing the plasma concentration of the filtered

ions. In part the action of colloids, digitalis glycosides and aminophylline is to increase glomerular filtration rate, hence to increase the filtered load of all ions. Ammonium chloride and calcium chloride, on the other hand, increase the plasma concentration of chloride at the expense of bicarbonate without increasing the plasma

TABLE VII

FUNCTIONAL CLASSIFICATION OF DIURETICS

A. *Physiological Diuretics*
   1. Diuretics which increase the filtered load of sodium and/or chloride ions.
      a. Colloids albumin, dextran, P.V.P., etc.
      b. Cardiac stimulants digitalis glycosides
      c Vasodilators of afferent glomerular arterioles aminophylline
      d. Acidifying agents· cation exchange resins, ammonium chloride
   2. Diuretics which more or less specifically antagonize over reabsorption of sodium and/or chloride ions
      a Aldosterone anti-secretory agents. amphenones
      b. Antialdosterone steroids SC5233, SC8109.
      c. Agents which increase the velocity of tubular flow in the most distal part of the nephron water.
      d. Agents which introduce a limiting ion gradient, osmotic diuretics mannitol, urea
B *Pharmacological Diuretics*
   1. Diuretics which specifically inhibit transport mechanisms for sodium and/or chloride ions
      a. Xanthines theophylline, theobromine, caffeine.
      b. Aminouracils aminoisometridine.
      c Mercurial diuretics· meralluride, mersalyl, etc.
      d. Chlorothiazide, hydrochlorothiazide.
   2 Diuretics which interfere with hydrogen for sodium exchange.
      a. Potassium salts KCl, $KNO_3$, etc.
      b. Carbonic anhydrase inhibitors acetazoleamide, chlorothiazide, dichlorphenamide.

concentration of sodium; hence these agents increase only the filtered load of chloride.

Antagonism of enhanced tubular reabsorption of sodium is most specifically accomplished by certain antialdosterone steroids which bind to renal tubular cells at those sites where aldosterone binds. These antisteroids displace aldosterone and reduce tubular reabsorption of sodium The aldosterone antisecretory agents, in con-

trast, depress the secretion of salt retaining hormone by the adrenal cortex. Water in large amounts impairs slightly the reabsorption of the last traces of sodium in distal tubules and collecting ducts, perhaps in part by increasing the velocity of flow and by diminishing the time of contact of the fluid with the tubular epithelium. Osmotic diuretics, such as urea, mannitol, hypertonic glucose, etc., limit the proximal tubular reabsortion of sodium and water and cause the delivery of excessive quantities of fluid and electrolytes into more distal parts of the nephron. Reabsorption distally is therefore incomplete. With the exception of the digitalis glycosides in congestive heart failure, the *Physiological Diuretics* exhibit a relatively low order of activity. At present they are most useful in supplementing other forms of therapy, although the antialdosterone steroids and antialdosterone secretory agents may well be harbingers of the diuretics of the future.

Diuretics, designated as *Pharmacological* in Table VII, include all of the potent inhibitors of tubular ion reabsorption. They correct glomerulo-tubular imbalance by reversibly inhibiting enzyme systems concerned with ion transport. They logically divide into two groups: one inhibiting sodium and chloride reabsorption, the other inhibiting sodium and bicarbonate reabsorption. There is reason to believe that at least three of the four classes of inhibitors of sodium and chloride transport have different mechanisms of action. In contrast, the sulfonamides all block the reabsorption of sodium and bicarbonate by virtue of their inhibition of carbonic anhydrase. Chlorothiazide has certain properties common to the two major groups of agents, in that it may block reabsorption of sodium and both chloride and bicarbonate ions.

*Use of Diuretics.* Diuretics are employed for the relief of generalized edema and find their greatest use in such chronic conditions as congestive heart failure, cirrhosis with ascites, and the nephrotic syndrome, including the nephrotic stage of nephritis and so-called genuine lipoid nephrosis. They are also useful in certain acute, self limited conditions such as toxemia of pregnancy, and in periodic recurrent edema, e.g., premenstrual edema. They are less frequently employed for dehydration in epilepsy and to reduce regional edema, e.g., that accompanying thrombophlebitis.

Diuretics other than water are of no value in reducing the nitrogen retention of chronic renal insufficiency, for they do not, in the usual sense, increase renal excretory functions other than that concerned with salt elimination. Under no circumstances should any diuretic be administered to an anuric or markedly oliguric patient, for none can initiate the flow of urine, and all are, in variable degree, toxic when retained in the body.

*Salt Restriction in Diuretic Therapy.* Since diuretics are employed primarily to establish a negative salt balance by promoting the excretion of sodium, the overall efficacy of therapy can be considerably enhanced by restricting the dietary intake of this ion. In fact, whenever diuretics are used, salt intake should be reduced at least to some extent. If the degree of salt privation is adequate, fluid restriction is unnecessary, inadvisable, and in fact, inhumane. An exception to this rule is the treatment of bromidism, where high salt and water intake, combined with mercurial diuresis, is employed to rid the body of bromide. In practice, it is often convenient to permit a moderate intake of salt in order to provide a more palatable diet and then to remove that salt from the body by diuretic therapy.

*Chapter VIII*

# COLLOIDS AS DIURETICS

EDEMA and/or ascites are commonly associated with and often roughly proportional to hypoproteinemia in cirrhosis, in the nephrotic state, and in protein malnutrition. In these conditions excessive transudation of fluid into the peritoneum and tissue interstices has in the past been explained solely or largely in terms of an *imbalance of forces across the capillary endothelium* created by low colloid osmotic (oncotic) pressure of the blood plasma Some have claimed that edema or ascites will accumulate if the plasma concentration of albumin is below 3 gm. per cent and that fluid will be absorbed into the vascular compartment if concentration is above this critical level. In cirrhosis, increased portal and intrahepatic capillary pressures constitute additional factors directing fluid into the peritoneum. Without in any sense detracting from the significance of hydrostatic and oncotic forces in determining transudation of fluid, it has become increasingly apparent that retention of salt and water in the edema and ascites of nephrosis and cirrhosis is no less dependent on altered renal function than it is in congestive heart failure (see Chapters III, V, and XII). Spontaneous diuresis has been observed to occur at a time when the concentration of plasma albumin is below 3 gm. per cent No diuresis may occur when plasma albumin is restored to a range significantly above the so-called critical level. It is not surprising, therefore, that the treatment of patients with nephrosis and cirrhosis with salt poor concentrated human albumin has not been uniformly successful in relieving edema and ascites. Hypoproteinemia is a contributory factor in edema and ascites; it is not a complete explanation of fluid retention in itself.

***Use of Albumin in Patients with Cirrhosis and Ascites*** has, with few exceptions, been unrewarding. Janeway observed no loss

of ascites in 6 patients with cirrhosis who received intravenously from 350 to 950 gm. of salt poor concentrated human serum albumin in daily doses of 50 gm. Thorn, Gibson, and Kunkel noted that occasional patients, after receiving numerous albumin infusions, exhibited diuresis and loss of ascites. Frequently patients with both edema and ascites lose interstitial but not peritoneal fluid in response to therapy. Patek observed that the concentration of protein in ascitic fluid increased in direct proportion to the increase in concentration in plasma during the intravenous infusion of albumin, an observation which is reasonable considering the relatively high permeability of liver capillaries to colloids. He noted that as much as 50 per cent of the administered albumin was transferred to the ascitic fluid. Although the oncotic effect of the proteins of plasma was restored to normal, ascites continued to collect at the usual rate. The intravenous administration of albumin increases plasma volume and no doubt elevates portal and intrahepatic capillary hydrostatic pressures as well. The combination of increased capillary pressure and increased colloid content of ascitic fluid offsets the beneficial effects of increased plasma protein. The administration of albumin and other colloids in cirrhosis with ascites is accordingly of little or no value.

*Use of Albumin in Patients with Nephrosis* has been somewhat more successful Janeway, Thorn, Luetscher, Riley, Siegal, Chinard, Eder, Lauson and their respective colleagues have observed diuresis and loss of edema in 50 to 70 per cent of selected patients receiving daily infusions of 25 to 75 gm. of salt poor human serum albumin. The albumin has usually been administered in 10 per cent solution in isotonic glucose over a period of 2 hr. or so. In the responsive patient, water diuresis follows each albumin injection. At first relatively little sodium and chloride are excreted. With repeated daily infusions, successive water diureses are accompanied by greater and greater salureses. It is interesting that the peak of each water diuresis precedes that of its accompanying saluresis 2 hours or more. With loss of salt, more profound water diuresis occurs, weight is lost and edema disappears. The most favorable responses occur in patients who exhibit no azotemia, and who have

normal or supernormal rates of glomerular filtration prior to therapy.

All who have measured plasma volume have found it increased following albumin infusion. However, Chinard has pointed out that the degree of expansion of volume is less than the theoretic iso-oncotic value. He suggests that, in consequence of the expanded plasma volume, capillary hydrostatic pressure rises. The outwardly directed filtering force increases, in part balancing the inwardly directed oncotic force and resulting in less than theoretic expansion of volume. Because of the very great permeability of the glomerular capillary membranes to protein in nephrosis, much of the albumin administered by vein is lost during the 24 hr. following the infusion. High cost of salt poor albumin and excessive urinary wasteage make this form of therapy impractical except on an experimental basis.

The water diuresis which almost immediately follows injection of albumin is no doubt related to expansion of plasma volume. Although the titre of ADH in plasma is primarily responsive to the osmolality of the body fluids, it is also affected by plasma volume or some derivative of volume (see Chapter V). According to Henry and Gouer, distension of the left atrium inhibits ADH release reflexly and causes water diuresis. Such atrial distension might well result from the injection of hyperoncotic albumin.

Eder and others have observed a high degree of correlation between increased rate of glomerular filtration and diuresis of water and salt in both spontaneous diureses and in those associated with the repeated infusions of albumin. These findings suggest that the glomerulo-tubular imbalance which underlies water and salt retention in nephrosis is due in some patients to a relative deficiency of glomerular filtration.

That enhanced tubular reabsorption plays some role in salt and water retention is equally evident. Burnett et al and Metcoff et al have shown that when PAH or thiosulfate are administered to nephrotic patients as sodium salts, they are largely excreted in combination with potassium. Normal subjects, in contrast, excrete them in the form in which they are given. Ingbar has shown that the administration of ACTH and cortisone causes the excretory

pattern of the normal subject to approximate that of the nephrotic. These facts, coupled with the observation of Luetscher and others that large amounts of aldosterone are excreted in the urine of patients with nephrosis, suggest that the glomerulo-tubular imbalance is in part due to enhanced exchange of sodium for potassium and no doubt for hydrogen and ammonia as well. However, in even greater degree, it is due to over-reabsorption of sodium and chloride as ion pairs. The infusion of albumin is associated with a reduction in urinary excretion of aldosterone, with increased sodium output, and with a decrease in body weight. The relative importance of increased filtration and of decreased tubular reabsorption of salt and water as causes of diuresis cannot be assessed at the present time. No doubt both are significant.

*Use of Dextran and Other Colloids in Nephrosis.* As was mentioned above, high cost and inadequate supply render therapy with salt poor human serum albumin impractical except on an experimental basis. Three colloids have been successfully substituted in the treatment of nephrosis: polyvinal pyrrolidone, gelatin, and dextran. In the past, acacia (gum arabic) has been used. The latter is mentioned here only to condemn it most strenuously, for as Hueper, Mannix and others have shown, its use leads to a storage disease known as arabinosis, characterized by splenomegally, hepatomegally, depressed formation of plasma proteins and other liver dysfunctions. Of the three recommended compounds, gelatin and dextran are most favored in this country. Polyvinyl pyrrolidone is retained in the body for long periods of time, and seems on this score to be less desirable. All of these colloids are essentially similar to albumin in their actions. When given to patients with nephrosis, they produce a transient expansion of plasma volume, water diuresis followed by salt diuresis, increase in glomerular filtration rate, and no doubt decrease in aldosterone excretion as well. These compounds are commonly given intravenously in 10 to 12 per cent solution in isotonic glucose. James et al recommend a dose of 1.2 to 1.8 gm. of dextran per Kg. body weight, infused at a rate of 2 to 4ml. per min. These authors suggest that blood pressure be measured every 20 min. and if systolic

normal or supernormal rates of glomerular filtration prior to therapy.

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## REFERENCES

1. EDER, H. A., LAUSON, H. D., CHINARD, F. P., GREIF, R. L., COLTZIAS, G. C. and VAN SLYKE, D. D.: A study of the mechanisms of edema formation in patients with the nephrotic syndrome. *J. Clin. Invest.*, *33*:636, 1954.
2. JAMES, J., GORDILLA, G., and METCOFF, J. Effect of infusion of hyperoncotic dextran in children with the nephrotic syndrome. *J. Clin. Invest*, *33*:1346, 1954
3. LUETSCHER, J. A. JR, DEMING, Q. B., JOHNSON, B. B., and PIEL, C. F.. Advances in management of the nephrotic state. *J. A. M. A.*, *153*: 1236, 1953.
4 PATEK, A. J., JR., MANKIN, H., COLCHER, H., LOWELL, A., and EARLE, D. P.: The effect of intravenous injection of concentrated human serum albumin upon blood plasma, ascites and renal functions in three patients with cirrhosis. *J. Clin. Invest.*, 27:135, 1948.
5. RILEY, C. M.: The management of the nephrotic syndrome. *Bull. New York Acad. Med.*, *28*:630, 1952.

pressure increases to values in excess of 140 mm. Hg, that the infusion be stopped.

Albumin, dextran and other colloids merely promote the elimination of fluid in nephrosis; they do not alter the course of the disease. Remission of edema is short lived and courses of therapy must be repeated at frequent intervals.

*Complications of Colloid Therapy.* All colloids may induce severe hypertension and convulsions or pulmonary edema when given rapidly and in large amounts to patients with anasarca, due no doubt to the sudden attraction of large volumes of fluid into the vascular system. It is therefore advisable to administer them in smaller than usual amounts in the presence of massive edema and pre-existing hypertension. Dextran prolongs bleeding time, due to inhibition of prothombin activation, and may be associated with epistaxis and/or subcutaneous ecchymosis. Certain patients are sensitive to dextran and may exhibit allergic manifestation following the first infusion. Others may develop minor or major sensitivity during repeated courses of therapy.

## SUMMARY

The intravenous administration of concentrated salt poor human serum albumin, of dextran or of gelatin to patients with nephrosis frequently induces water diuresis and subsequently saluresis and loss of weight. Repeated daily infusions are necessary to cause a significant response, for all colloids are rapidly eliminated in the urine in consequence of excessively high permeability of the glomerular membranes Plasma volume is expanded, glomerular filtration rate and renal blood flow are increased, and urinary excretion of aldosterone is reduced. Diuresis and loss of weight are results of both increased filtration and reduced tubular reabsorption of salt and water. Colloid therapy of cirrhosis with ascites is commonly ineffective due to rapid transfer of the oncotic agent into ascitic fluid. Since portal and intrahepatic capillary hydrostatic pressures increase with increased plasma volume, and since the colloid is transferred to ascitic fluid, no significant reabsorption of fluid from the peritoneum occurs, even though the colloid osmotic pressure of the plasma is increased to normal levels.

crease. However, certain patients exhibit significant diuresis without appreciable changes in these discrete renal functions. One such study on a patient in congestive failure is summarized in Figure 24. The administration of equivalent doses of digoxin to normal subjects and to patients with cirrhotic and nephrotic edema produced a perceptible but less significant diuresis of salt and water with no change in renal blood flow or filtration rate. Farber's observations

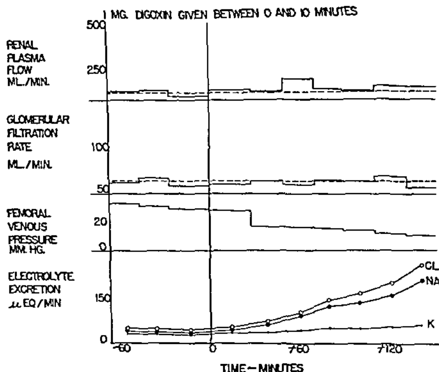



Fig. 24. The diuretic effects of the intravenous administration of 1.0 mg of Digoxin in a patient in congestive failure. Increased excretion of sodium and chloride in the absence of change in renal plasma flow and glomerular filtration rate suggests a direct effect of the glycoside on the kidneys. (From S J. Farber, J.D Alexander, E.D. Pelligrino, and D.P Earle *Circulation*, 4.378, 1951 )

strongly imply that at a constant filtered sodium load, reabsorption is slightly depressed and excretion slightly increased by a direct action of digoxin on the renal tubules. The effect is more marked in patients in congestive failure than in those with nephrotic or

*Chapter IX*

# DIGITALIS GLYCOSIDES

A NUMBER of plant and animal extracts containing cardiac glycosides have been employed as folk remedies over the centuries. Squill was known to the ancient Egyptians and was used by the Romans as a diuretic, heart tonic, emetic and rat poison. Dried toad skin, containing the glycoside bufagin, was both a Chinese and Western folk medicine. Digitalis or foxglove, while long used by the Welsh, became popular as a treatment of dropsy following the classic description of its actions by William Withering in 1785. Withering was aware that digitalis was not equally effective in all forms of dropsy, but apparently did not recognize that his successes were restricted to patients with heart disease. John Ferriar in 1799 was the first to ascribe to digitalis a primary cardiac action and to relegate to a secondary position its diuretic effects.

Today the cardiac glycosides are not generally regarded as diuretics in the true sense of the word. The author has chosen to classify them as physiological diuretics, implying that they restore the work capacity of the heart and counteract the circulatory deficiencies which lead to glomerulo-tubular imbalance and to compensatory retention of salt and water. It is widely accepted that the cardiac glycosides have no place in the therapy of edema other than that associated with congestive circulatory failure. The actions of these drugs on the heart and circulation which secondarily result in diuresis and discharge of edema fluid are outside the scope of this monograph.

*A Direct Diuretic Action of Cardiac Glycosides* has recently been described. Farber and his associates noted that the intravenous administration of 1.0 to 1.5 mg. of digoxin to patients in congestive failure causes a prompt diuresis of sodium and water. Frequently renal blood flow and rate of glomerular filtration in-

action could be ascribed to suppression of aldosterone stimulation of tubular reabsorption.

However, another interpretation is possible. Schatzmann first showed in 1953 that strophanthin prevents the uptake of potassium and the elimination of sodium that normally occurs when cold stored red cells are incubated with glucose at 37°C. The drug does not affect oxygen consumption nor lactic acid production, hence inhibits ion transport beyond the stage of energy production. Joyce and Weatherall and Kahn and Acheson showed that other cardiac glycosides including digoxin exert the same effect. Glynn has presented more direct evidence that the cardiac glycosides interfere with the ion pump rather than its energy source. Neither desoxycorticosterone nor aldosterone modify the action of digoxin on red cells.

It is impossible at the moment to decide which thesis applies to the renal tubule, or in fact whether either does. Furthermore, it is impossible to assess the relative significance of the direct renal action and of the cardiac stimulating action of the digitalis glycosides in explaining their diuretic effects in pati...
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*on the heart* *The* unsaturated lactone *portion is* necessary and either *splitting* the ring or saturating it destroys activity. *The* steroid nucleus bears a superficial resemblance to *desoxy*corticosterone and aldosterone. One might speculate *that digoxin* binds to the same receptor sites of renal tubules which bind aldosterone, displacing *the* salt retaining *steroid* from its combination *Digoxin* could then be classified as an *anti*aldosterone and its *direct* diuretic

action could be ascribed to suppression of aldosterone stimulation of tubular reabsorption.

However, another interpretation is possible. Schatzmann first showed in 1953 that strophanthin prevents the uptake of potassium and the elimination of sodium that normally occurs when cold stored red cells are incubated with glucose at 37°C. The drug does not affect oxygen consumption nor lactic acid production, hence inhibits ion transport beyond the stage of energy production. Joyce and Weatherall and Kahn and Acheson showed that other cardiac glycosides including digoxin exert the same effect. Glynn has presented more direct evidence that the cardiac glycosides interfere with the ion pump rather than its energy source. Neither desoxycorticosterone nor aldosterone modify the action of digoxin on red cells.

It is impossible at the moment to decide which thesis applies to the renal tubule, or in fact whether either does. Furthermore, it is impossible to assess the relative significance of the direct renal action and of the cardiac stimulating action of the digitalis glycosides in explaining their diuretic effects in patients wth congestive circulatory failure.

## SUMMARY

The cardiac glycosides have a clinically significant diuretic action only in congestive heart failure and presumably exert their major effects through the improvement in cardio-circulatory dynamics which they induce. However, these drugs apparently depress renal tubular reabsorption of sodium chloride in modest degree. It is possible that this action is antialdosterone in nature or that the drugs block ion pumps of renal tubular cells more directly. These findings should not be interpreted as justification for use of presently available glycosides in edemas other than those resulting from congestive heart failure.

## REFERENCES

1. Farber, S. J., Alexander, J. D., Pallegrino, E. D., and Earle, D. P.: The effects of intravenously administered digoxin on water and electrolyte excretions and on renal function. *Circulation, 4:* 378, 1951.

cirrhotic edema or in normal non-edematous controls. If filtration rate increases in patients in congestive failure, the diuresis may be much more profound.

Hyman and his associates have recently demonstrated that the injection of 0.065 to 0.250 mg. of digoxin into one renal artery of an anesthetized dog produces diuresis of sodium and water which is restricted to the side of drug administration and which lasts for the 2 hr. period of observation. A greater response was obtained in one dog in congestive failure due to experimental mitral stenosis. Barger, in an as yet unpublished study, has extended these observations on unanesthetized dogs in congestive failure with ureters separately explanted in the abdominal wall and with a catheter fixed in one renal artery. Injection of 0.5 mg. of digoxin into one renal artery results in diuresis of sodium and water, most apparent on the side injected and lasting for more than 24 hr. Shatzmann, Windhager and Solomon have demonstrated that perfusion of the proximal tubule of Necturus with solutions containing minute amounts of ouabain inhibits the active transport of sodium. These several investigations are all consonant with the view that cardiac glycosides can inhibit tubular transport of sodium by a direct action on the kidney.

*The Mechanism of Diuretic Action of Digoxin* in the studies of Farber, Hyman, Barger, and Schatzman is by no means clear, but one may speculate along the following lines. Cardiac glycosides are complex molecules consisting of three basic components: a sugar moiety or glycone, a 5 or 6 membered unsaturated lactone ring, and a cyclopentanophenanthrene nucleus or steroid moiety. While it influences solubility and duration of action, the glycone is not necessary for the molecule to exhibit its characteristic effects on the heart. The unsaturated lactone portion is necessary and either splitting the ring or saturating it destroys activity. The steroid nucleus bears a superficial resemblance to desoxycorticosterone and aldosterone. One might speculate that digoxin binds to the same receptor sites of renal tubules which bind aldosterone, displacing the salt retaining steroid from its combination. Digoxin could then be classified as an antialdosterone and its direct diuretic

*Chapter X*

# ACIDIFYING AGENTS

## CATION EXCHANGE RESINS

In the introduction to this section, it was pointed out that diuretics are administered to induce a negative balance of sodium, and that some restriction of sodium intake is imperative, if one is to attain this end. While preparation of a nutritious and palatable diet containing 2 to 3 gm. of salt per day is perfectly feasible, limitation of intake to 1.0 gm or less per day is virtually incompatible with a diet of adequate nutritive content and taste. Dock in 1946 first suggested that ion exchange resins, long used in industry for purposes of de-salination, might be administered to patients to prevent intestinal absorption and to promote fecal excretion of sodium. Not only might such resins permit a more liberal intake of sodium, they might also be useful to abstract sodium from the body by an enteric rather than by a renal route. The first of these ends is more frequently achieved in practice than is the second.

*Chemical Nature of Ion Exchange Resins.* An ion exchange resin is a crosslinked polymer which contains either acidic or basic groups and which, therefore, can exchange either cations or anions with the surrounding fluid medium. The resin has essentially infinite molecular size and its monomeric components are extensively crosslinked with stable carbon bridges. Accordingly, it is highly insoluble. The resins, useful clinically for removal of sodium from the gut, contain sulfonic, carboxylic or phenol acid radicals. They may be classified as phenol-formaldehyde, phenol-methylene, or polystyrene polymers. Those which exchange anions are polyamines and are sometimes added in small amounts to the cation exchange resins to reduce their acidic properties. The gen-

2. Glynn, I. M.: The action of cardiac glycosides on sodium and potassium movements in human red cells. *J. Physiol., 136*:148, 1957.
3. Hyman, A. L., Jacques, W. E., and Hyman, E. S.: Observations on the direct effect of digoxin on renal excretion of sodium and water. *Am. Heart J., 52*:592, 1956.
4. Schatzmann, *H. J.*: Herzglykoside als Hemmstoffe fur den aktiven Kalium and Natrium Transport durch die Erythrocytenmembran. *Helvet. physiol. acta, 11*:346, 1953.
5. Schatzmann, H. J., Windhager, E. E., and Solomon, A. K.: Studies in single proximal tubules of the Necturus kidney. *II.* Effect of 2, 4-dinitrophenol and ouabain on water reabsorption. *Am. J. Physiol.*, to be published.

the ammonium chloride or potassium chloride formed is absorbed in the lower part of the digestive tract. In the less acid environment of the intestine, the hydrogen cycle resin is converted to sodium, potassium, calcium and magnesium cycles and excreted as such in the feces.

Theoretically, at the pH and concentration of the lower intestine, some 6 to 7 mEq. of cations should be bound to each gm. of resin Actually much less is bound; only 0.9 to 1.2 mEq. of sodium, ± 1.0 mEq. of potassium, and considerably smaller but by no means negligible amounts of calcium and magnesium are bound per gm. of resin. This failure to bind theoretical quantities is in part a result of very slow attainment of equilibrium due to the compact structure of the resin lattice. It is also probable that protein, amino acids, etc., inhibit ion uptake. As will be explained below, the colonic mucosa specifically abstracts sodium from the resin, reducing the quantity bound.

***Binding of Ions in the Intestinal Lumen.*** From the work of Visscher and his associates on the dog, it is evident that considerable quantities of sodium exchange in both directions across the intestinal epithelium between blood stream and luminal contents during the transit of food and fluid along the intestine. Exchange proceeds at such a rate that the sodium present in the blood turns over or exchanges with that in the gut once every 90 min. This means that in a 70 Kg. man, 190 gm. of sodium is delivered into and abstracted from the gut each day. If one introduces into this stream of sodium 50 to 100 gm. of resin, it is remarkable that fecal excretion is not highly significant and that sodium is not rapidly abstracted from the body, especially in edematous patients maintained on low salt diets.

Visscher has demonstrated that the rate of exchange of sodium across the duodenal epithelium is high, and that both rate of transfer into the gut and out of the gut diminish through jejunum, ileum and colon. However, the ratio of rate of transfer into blood/rate of transfer into lumen increases progressively from oral to aboral ends of the intestine. The greater this transfer ratio, the more completely is sodium cleared from the lumen of the gut. The colon is a highly efficient absorber of sodium and the feces

eral structural pattern of a carboxylic cation exchange resin is illustrated in Figure 25.

Resins, like all other dissociated compounds, must obey laws of ionic equivalence. Figure 25 shows the several anionic sites of the resin neutralized with $Na^+$, $H^+$, $NH^+_4$ and $K^+$. These anionic

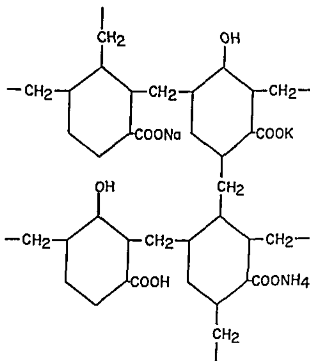


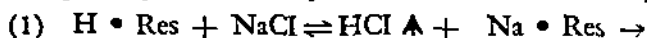

Fig. 25. Structure of a carboxylic cation exchange resin.

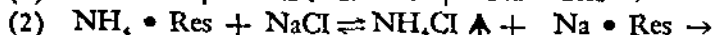
sites have differing affinities for the several ions found in the gut contents. Were all present in the same concentrations, the quantities of ions bound would be in the order $Ca^{++} > Mg^{++} > K^+ > Na^+$. However, the concentration of sodium in the gut considerably exceeds that of any other ion. Hence when the so-called hydrogen cycle resin (all anionic sites neutralized with $H^+$ ions) is administered, sodium is bound in greatest quantity. The binding of ions by carboxylic resins is especially dependent on pH. In the highly acid environment of the stomach, ammonium or potassium cycle resins are converted to the hydrogen cycle, and

ably and excrete more sodium in the feces than is contained in the diet; they lose weight. Presumably their colonic mechanisms are less intensely stimulated to conserve salt.

Potassium is contained in digestive secretions in much higher concentration than in extracellular fluid, i.e., as much as 15 to 20 mEq per liter. When a resin is administered in its hydrogen cycle, it binds nearly as much potassium as sodium If sodium is excessively conserved due to adrenal stimulation, considerably more potassium than sodium may be eliminated. This results from the high affinity of resins for potassium, the relatively high concentration of potassium in digestive secretions, and perhaps, under adrenal stimulation, to an active exchange of potassium for sodium by the colonic mucosa. Significant quantities of calcium and magnesium are also bound by resin. The possibility exists that resins may also remove trace metals, riboflavin, and thiamine.

***Alterations in Composition of the Body Fluids Induced by Resins*** derive directly from the ion binding properties described above. Hydrogen cycle and ammonium cycle resins induce hyperchloremic metabolic acidosis. The plasma bicarbonate concentration decreases from its normal level of 26 to 28 mEq. per liter to between 10 and 20 mEq. per liter The plasma chloride concentration increases from its normal level of 105 to as high as 115 to 120 mEq. per liter. Ordinarily, plasma sodium concentration is unchanged or is only slightly depressed. Plasma pH decreases moderately. These manifestations of acidosis produced by the ingestion of 45 gm. of hydrogen or ammonium cycles resin per day are comparable to those produced by 7 to 10 gm. of ammonium chloride per day. In fact they are the exact chemical equivalent.

(1) 
$H \cdot Res + NaCl \rightleftharpoons HCl \uparrow + Na \cdot Res \rightarrow$

(2) 
$NH_4 \cdot Res + NaCl \rightleftharpoons NH_4Cl \uparrow + Na \cdot Res \rightarrow$

As shown in equation (1), hydrogen cycle resin (H·Res) reacts with NaCl in the gut to form HCl, which is absorbed and neutralized in the body by intracellular and extracellular buffers, including bicarbonate. As shown in equation (2), ammonium cycle resin ($NH_4$·Res) reacts with NaCl to from $NH_4Cl$. This salt is absorbed, converted into urea and HCl in the liver, and the acid so formed is neutralized by body buffers. While the patient with

of man normally contain negligible amounts of this ion. It is probable that resin binds sodium in the oral end of the intestine and that sodium is removed and replaced with potassium and, to a slight extent, hydrogen in the aboral end.

The activity of the colon in removing sodium from feces and from resin is regulated by salt retaining adrenal cortical steroids, probably by aldosterone. Berger and his associates have shown that the rat, an animal which normally excretes fair amounts of sodium in the feces, completely absorbs this ion when treated with desoxycorticosterone. Normal human subjects, excreting significant amounts of sodium bound to resin, reduce fecal excretion essentially to zero when treated with DOCA. Emerson et al have shown that adrenalectomized patients or patients with Addisons disease who lack aldosterone excrete far more sodium in the feces when given resin than do normal subjects.

It has been general clinical experience that patients vary widely in their response to resins. Some excrete small amounts of sodium bound to resin, even less than the gm. or so contained in a very low sodium diet. Others may excrete greater amounts of sodium than are present in the food ingested. However, they rarely develop a significant negative balance. These findings have been loosely explained by the statement that the resin reacts differently with exogenous and endogenous sodium, i.e., that resins will remove a limited amount of dietary sodium in the feces, but that they will not abstract sodium from body reserves. In view of the rapid turn-over of sodium across the gut wall, this explanation is patent nonsense, if it is interpreted literally. A much more reasonable explanation is that the efficacy of resins in removing sodium from the body is inversely related to the intensity of stimulation of salt retaining mechanisms. If the adrenal salt retaining system is maximally activated, the concentration of sodium in sweat, saliva, urine, and colonic contents will be minimal. Essentially all of the sodium bound by resin in the upper gut will be abstracted in the colon. Restriction of dietary sodium intake stimulates sodium conservation by both normal subjects and by edematous patients. Liberalization of dietary intake reduces conservation and permits fecal loss of sodium on resin. Some patients respond more favor-

consideration of ammonium chloride therapy. (2) Compensation for diversion of ingested ions from a urinary to a fecal route of elimination Commonly on institution of resin therapy, urinary sodium excretion decreases. If the decrease in renal excretion is equal to the increase in fecal excretion, no net loss of body sodium occurs. Occasionally, however, urinary output of sodium is less markedly depressed; the sum of urinary and fecal excretions exceeds intake, and modest loss of weight occurs. Less frequently a significant diuresis of chloride, sodium and water results from the acidosis, and edema clears. In contrast, urinary potassium excretion is rarely reduced to a degree sufficient to balance fecal loses when ammonium or hydrogen cycle resins are given; hence body stores of potassium may be rapidly depleted.

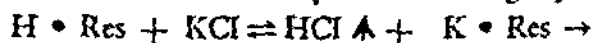
*Toxicity.* Cation exchange resins are by no means benign therapeutic agents. The sulfonic resins cause epigastric burning and discomfort due to their relatively strong acid properties and have now been replaced by carboxylic resins. These latter, though less irritating, may produce a sense of fullness, and rarely nausea and vomiting. The most common complaint is constipation, a difficulty which may be alleviated by administration of methylcellulose or other hydrophilic bulking agents. However, abdominal distension and constipation are frequently signs of potassium depletion and should be considered as such till proven otherwise. Fecal impaction is a possible hazard, especially in the elderly.

The mild to moderate acidosis which accompanies resin therapy is of little significance if the patient has normal renal function. However, increasing dyspnea, azotemia, and oliguria may be signs of severe acidosis in patients with inadequate renal reserve Weakness, lassitude, abdominal distension, constipation, tachycardia, and cardiac irregularities are signs of significant potassium depletion and may occur, although rarely, when the patient is receiving a part of the resin dose in the potassium cycle.

In acute renal failure potassium cycle resins are absolutely interdicted, although hydrogen cycle or sodium cycle resins are occasionally used to combat hyperpotassemia. The hydrogen cycle resins increase acidosis and are to be avoided if the patient is severely acidotic, the sodium cycle resins increase extracellular ion

normal renal function can and does compensate this acidosis and keep it within reasonable bounds, the patient with severely reduced renal function cannot. Such individuals may become extremely acidotic, exhibit Kussmaul breathing and become oliguric, azotemic, and comatose on prolonged resin therapy.

A somewhat more subtle disturbance produced by hydrogen and ammonium cycle resins is hypokalemia and depletion of tissue stores of potassium as indicated by the following equation.



$$H \bullet Res + KCl \rightleftharpoons HCl \uparrow + K \bullet Res \rightarrow$$

The degree of reduction of plasma concentration of potassium is unfortunately not an accurate indication of the extent of depletion of body potassium reserves, for large amounts of this ion may be removed from tissues with relatively minor alterations *in plasma level* Signs and symptoms referable to potassium depletion are discussed in Chapter XIX. All resins now marketed for removal of sodium in the gut contain roughly one-third of total resin in the potassium cycle. In the absence of vomiting, diarrhea or anorexia, this quantity of potassium is sufficient to protect the patient from excessive potassium loss. Administration of resin entirely in the potassium cycle would cause no change in acid base balance. One might, therefore, presume this to be the ideal form in which to administer resins. However, the incorporation of even one-third of total resin in the potassium cycle reduces overall efficacy of binding of sodium. Unfortunately, no alternative exists but to supply a part of the resin in the potassium cycle or to give potassium supplements; the two are equivalent.

Hypocalcemia has been no problem except in patients maintained on daily doses of resin for 6 months or more. Oral calcium supplements are of little value, for this ion is strongly bound to resin. Calcium in the form of the gluconate can be administered parenterally or resin can be given in interrupted courses with the view that calcium deficits can be made up during the drug-free interval.

*Renal Responses to Resin Therapy* fall into two general categories: (1) Compensations for hyperchloremic metabolic acidosis, namely low urine pH and high rate of excretion of titratable acid and ammonia. These factors will be discussed in Chapter XI in a

therapy, rendering more effective subsequent treatment with mercurial compounds. It can scarcely be considered a prime reason for the administration of resins; the same effect can be obtained at considerably less expense by administering ammonium chloride. According to this view, resins should be considered as adjuvants in the treatment of edema, not as primary therapeutic agents.

Others have had greater success with resins in the primary removal of edema fluid. They have noted in favorable cases that fecal elimination of sodium may exceed dietary intake and that diuresis of sodium, chloride and water may lead to rapid reduction of edema. Still others complain that relatively little liberalization of dietary sodium is possible without gain of weight and that resins are relatively ineffective in the primary removal of edema fluid. Resins have been employed with success and with failure in congestive heart failure, cirrhosis with ascites, nephrosis and the nephrotic stage of glomerulonephritis, pre-eclampsia and a variety of other diseases. The author would reconcile these conflicting views in the following terms. The adequacy of response to resin therapy is less determined by the nature of the primary disease process than by its severity as expressed in the intensity of stimulation of salt conserving mechanisms. The more intense the stimulation of salt conservation, the more avid the absorption of sodium by the colon, the more complete the reabsorption of sodium in the renal tubule and the less the fecal and urinary excretion of salt and water. When salt retention is less actively stimulated, fecal loss of sodium on resin is greater and the diuresis in response to acidosis is more significant. Whether a given patient will respond must be determined by therapeutic trial.

*Contraindications.* Resins should not be used in severe renal disease because of the danger of marked acidosis and its attendant oliguria and azotemia Potassium and ammonium cycle resins are absolutely interdicted in acute renal failure and possible gain from the use of hydrogen and sodium cycle resins in removing potassium from the body must be balanced against the acidosis and edema which they induce. Ammonium cycle resins should not be employed in patients with severe liver insufficiency because of the possibility of ammonia toxicity. Adequate potassium intake must

reserves and are to be avoided if the patient has been overhydrated with saline early in the course of his disease.

Patients maintained on a low sodium diet, given daily doses of resins and subjected to repeated mercurial diuresis may develop the low salt syndrome, i.e., dilutional hyponatremia. This condition is discussed in Chapter XIX. When resins are given daily for long periods of time, hypocalcemia, muscle cramps and latent tetany may develop. Digitalis toxicity in the course of resin therapy is commonly associated with potassium depletion.

*Dosage and Route of Administration.* Resins are best given by mouth, but can be given per rectum in acute renal failure if nausea and vomiting make the oral route impractical. Resins are commonly given in amounts of 30 to 60 gm. per day in divided doses with meals. They are moderately unpleasant in taste and texture but can be adequately masked by suspension in fruit juices or tomato juice or by mixing in mashed potatoes or apple sauce. They may be given daily, or in interrupted courses of 4 days of drug, 3 days drug free.

A variety of preparations of carboxyl resins are available. Resodec is a mixture of two-thirds ammonium and one-third potassium cycle resin. Carboresin is a mixture of 88 per cent carboxyl resin and 12 per cent polyamine resin. Of the 88 per cent carboxyl resin, two-thirds is in the hydrogen cycle, one-third in the potassium cycle. Evidence that such amounts of polyamine resin significantly reduce acidosis is inconclusive. For potassium removal in acute renal failure, pure sodium cycle and pure hydrogen cycle resins are available.

*Clinical Use of Cation Exchange Resins.* Opinions differ as to the role of cation exchange resins in the therapy of edematous patients. A rather conservative view is that potent diuretics should be administered to effect the elimination of edema fluid. Thereafter resins and moderate dietary salt restriction may be employed to keep the patient edema free. In favorable cases, salt intake may be liberalized to such an extent that an easily prepared, nutritious and palatable diet is possible. Since resins induce a hyperchloremic metabolic acidosis, they potentiate the action of organomercurial diuretics. This can be considered as a useful by-product of resin

disturbances and clinical symptoms during prolonged exchange resin therapy in congesitve heart failure. *Am. J. Med.*, *14*:391, 1953.

5. Weston, R. E., Grossman, J., Borum, E. R., Guerin, H. A., Mark, H., Ullman, T. D., Wolfman, M., and Leiter, L.: Metabolic studies on the effects of ion exchange resins in edematous patients with cardiac and renal disease. *Am. J. Med.*, *14*:404, 1953.

also be assured. Elevation of blood ammonia and potassium depletion are contributory factors in precipitation of liver coma.

## SUMMARY

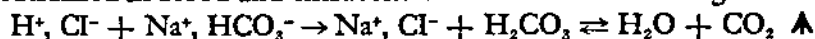
Cation exchange resins are macromolecular polyanionic lattices, insoluble and non-absorbable. When introduced in the digestive tract in hydrogen, ammonium and potassium cycles, they exchange these ions in variable degree for sodium present in the digestive secretions. At best their sodium binding capacity in the gut is low, and large doses must be administered to extract appreciable quantities of this ion. Hydrogen and ammonium cycle resins produce hyperchloremic metabolic acidosis, and since they preferentially bind potassium, tend to deplete body stores of this ion. Administration of one-third of the total resin dose in the potassium cycle in most instances protects against a negative potassium balance.

In favorable cases, the administration of resins abstracts sufficient sodium in the feces to permit some liberalization of intake without gain in weight. This somewhat simplifies preparation of a diet, and makes possible one of greater palatability and nutritive value. In some edematous patients, the acidosis induced by the resin causes primary diuresis and loss of weight. Resins, like other acidifying agents, potentiate mercurial diuresis. In some patients, maximally conserving sodium, resins abstract negligible quantities of this ion in the feces, instead abstract potassium. Adequacy of therapeutic response is largely dependent on degree of adrenal steroid stimulation of a colonic mechanism which reabsorbs sodium from the feces in exchange for potassium.

## REFERENCES

1. Ion Exchange Resins in Medicine and Biological Research. *Ann. New York Acad. Sc.*, *57:61*, 1953.
2. DOCK, W.: Sodium depletion as a therapeutic procedure. The value of ion exchange resins in withdrawing sodium from the body. *Tr. A. Am. Physicians*, *59:282*, 1946.
3. DUNCAN, L. E.: Treatment of edema by removal of body sodium by a cation exchange resin. *Am. J. Med.*, *14:425*, 1953.
4. GREENMAN, L., SHALER, J. B., and DANOWSKI, T. S.: Biochemical

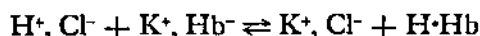
buffers contribute to the neutralization of strong acid in the body; the most familiar is bicarbonate. Hydrochloric acid is in part neutralized in blood and extracellular fluid in the following manner:



$$H^+, Cl^- + Na^+, HCO_3^- \rightarrow Na^+, Cl^- + H_2CO_3 \rightleftharpoons H_2O + CO_2 \uparrow$$

The net result is that a strong highly ionized acid, $H^+$, $Cl^-$, is converted into a weak un-ionized acid, $H_2CO_3$. Of equal importance, this weak acid is dehydrated to $CO_2$ and excreted by the lungs. The strong acid has completely disappeared, along with some fraction of the bicarbonate, and an equivalent amount of neutral sodium chloride has taken its place. Incidently pH decreases, but not in proportion to the fall in bicarbonate concentration, for breathing is stimulated. The administration of ammonium chloride therefore depresses plasma pH and bicarbonate concentration and elevates plasma chloride concentration, i.e., produces hyperchloremic metabolic acidosis.

A second buffer system of significance is hemoglobin. Hemoglobin exists in part as an ionized salt, potassium hemoglobinate, in part as a weak un-ionized acid, hemoglobin. Hydrochloric acid penetrates and is neutralized within erythrocytes in the manner described by the following equation:



$$H^+, Cl^- + K^+, Hb^- \rightleftharpoons K^+, Cl^- + H{\cdot}Hb$$

Again a strong acid has disappeared to be replaced by a weak acid. The reaction depicted by this equation is dependent on the general buffer properties of hemoglobin, not on its respiratory function in the transport of oxygen and carbon dioxide. To a slight extent the plasma proteins play a similar role, but since they are present in lower concentration than hemoglobin, their contribution is less significant.

It is a common misconception that the major part of an administered acid load is neutralized by blood buffers. Actually nothing could be further from the truth. As Van Slyke and Cullen pointed out many years ago and as Swan, Schwartz and others have recently emphasized, blood buffers neutralize but 15 to 20 per cent of an administered acid load; interstitial fluid buffers, largely bicarbonate, neutralize 30 to 35 per cent; and fully 50 per cent is neutralized by cellular buffers and by bone.

*Chapter XI*

# ACIDIFYING AGENTS

## AMMONIUM CHLORIDE

THE diuretic properties of acidifying salts were first described by Schultz in 1918. However, our present appreciation of the alterations in acid base balance and ionic structure of body fluids which these agents induce and which account for their diuretic action is based on the classic studies of Haldane, Gamble, Loeb, Keith and others. Ammonium chloride and nitrate, calcium chloride and nitrate, and hydrochloric acid have all been administered as acidifying agents. The nitrates have been discarded because they occasionally cause methemoglobinemia as a consequence of their reduction to nitrite in the gut. Calcium salts have been discarded because they frequently cause epigastric distress, abdominal discomfort, constipation, malaise, and muscle cramping. Ammonium chloride remains as the agent of choice, although cation exchange resins and acetazoleamide may also be employed to acidify the body fluids.

Ammonium chloride is rapidly absorbed in the gut, transported to the liver by the portal circulation and there converted to urea and hydrochloric acid. The net effect on acid base balance is the same as though an equivalent amount of hydrochloric acid had been ingested. The calcium ion of calcium chloride is poorly absorbed; most of a large oral dose is excreted in the feces as insoluble carbonate and phosphate. The chloride ion is readily absorbed in exchange for bicarbonate. The net effect again is equivalent to the ingestion of hydrochloric acid.

*Neutralization of Acid in the Body.* According to Van Slyke, the normal human body contains enough buffer to neutralize 1000 mEq. of hydrochloric acid before the pH of cells and extracellular fluid decreases to a level incompatible with life. A variety of

each day in divided doses. Chloride excretion rose sharply on the first day. Although the excretion of ammonia and titratable acid increased modestly, nearly all of the excess chloride in the urine was neutralized by sodium withdrawn from body reserves. During the subsequent 4 days of acid ingestion, progressively less and less

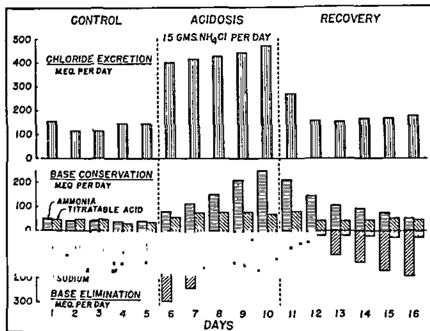



Fig 26 Effects of an acid load (15 gm per day of ammonium chloride) on the excretion of sodium, potassium, chloride, ammonia and titratable acid in normal man. (Drawn from data of O W Sartorius, J C Roemmelt, and R.F. Pitts: *J Clin. Invest*, 28 423, 1949)

sodium was sacrificed to neutralize chloride. Instead, on the second and third days, potassium, withdrawn from cellular stores, was utilized in increasing amounts. However, during the last 2 days the major fraction of the urinary chloride was eliminated in combination with ammonia. In fact, this mechanism permitted the achievement of acid base equilibrium and halted the loss of sodium and potassium by the fifth day of acid ingestion.

The loss of appreciable quantities of sodium and potassium from extracellular and cellular reserves is associated with the

Most cells, other than erythrocytes, can be described as effectively impermeable to chloride ions. Therefore, hydrochloric acid per se cannot penetrate to be neutralized by cellular buffers, i.e., by protein and organic phosphate complexes. However, cells are permeable to $H^+$ ions and to $Na^+$ and $K^+$ ions. Cells share their buffering powers with extracellular fluid by permitting the entrance of $H^+$ ions in exchange for equivalent numbers of $Na^+$ and $K^+$ ions which leave the cells. The chloride of hydrochloric acid remains extracellular.

$$2H^+ + K^+, Pr^- + Na^+, \text{Org } PO_4^- \rightleftharpoons H \cdot Pr + H \cdot \text{Org } PO_4 + Na^+ + K^+$$

Ammonium chloride therefore causes the loss of both potassium and sodium ions from cells and the gain of hydrogen ions. Accordingly the *pH* of the cell contents decreases. Since hydrogen ions bound to intracellular buffers are less ionized than were the sodium and potassium ions originally present, the osmotic pressure is reduced and water is lost by osmosis. According to Bergstrom and Wallace bone shares a part of its buffer capacity with extracellular fluid in much the same manner as do cells, giving up sodium ions in exchange for hydrogen ions.

*Renal Compensations for Acid Loading.* The study of Sartorius and Roemmelt, summarized in Figure 26, describes the alterations in renal excretion of ions in a normal individual over a five day period, during which 15 gm. of ammonium chloride were ingested each day. For the entire 16 day course of the experiment, the subject was maintained on a diet of constant composition with respect to electrolytes and calories. During the first 5 days, which constituted the control period, the subject excreted an average of 130 mEq of chloride, 125 mEq. of sodium, and 78 mEq. of potassium. Because the diet, like all normal diets, had an acid ash residue, 40 mEq. of ammonia and 40 mEq. of titratable acid were eliminated each day to balance the acid base budget. Ammonia and titratable acid are plotted upwards from the base line across the lower part of the chart to indicate that they represent cation conservation Sodium and potassium are plotted downward from the same base line to indicate cation loss.

During the second 5 day period, 15 gm. of ammonium chloride, equivalent to 280 mEq of hydrochloric acid, was administered

creasing quantities, until by the fifth day it neutralized all of the excess urinary anion. The ammonia excretory mechanism is the only truly compensatory one, in that it protects all cation reserves of the body. The potassium exchange mechanism, however, permits the participation of the large buffer reserves of cells in the homeostasis of body neutrality. The loss of ions from cellular and extracellular compartments is accompanied by a nearly equivalent loss of water. However, loss of water and decline in body weight are not strictly proportional to loss of ions, for plasma sodium concentration and total osmolality decrease somewhat.

The gradual increase in ammonia excretion under acid loading is a well documented observation of considerable significance. Because ammonia output increases slowly, body sodium is lost during the first few days of acid therapy. Because ammonia output rises to equal the acid load within 3 to 5 days, the diuretic response is brief. Nothing is gained by prolonged ammonium chloride therapy, the drug should be given in interrupted courses of 3 to 4 days, separated by equal or longer drug free intervals. The finding of rapid restoration of body sodium stores when ammonium chloride is withheld, emphasizes the fact that sodium intake must be severely restricted, if the losses sustained during acid loading are to be maintained in the drug free interval.

Davis and Yudkin, Rector, Seldin *et al.*, and Leonard and Orloff have shown that the gradual increase in rate of excretion of ammonia with acid loading is at least in part due to an adaptive increase in the activity of the enzyme, glutaminase, in the renal tubular cells. This enzyme catalyzes the deamidation of glutamine to glutamic acid; hence increases the rate of production of ammonia. The ammonia diffuses into acid urine where it is trapped as ammonium ion. As pointed out in Chapter IV, buffering of hydrogen ions by ammonia permits continued exchange of hydrogen for sodium. An increased rate of ammonia production obviously favors the salvage of sodium by distal tubules.

The stimulus for the adaptive increase in glutaminase activity might be either an increase in acidity of tubular cells or a reduction in body sodium stores. That the latter might be the more significant factor is suggested by the findings of Schwartz et al. They

excretion of nearly equivalent quantities of water and reduction in body weight. Normal individuals on such acidifying regimens lose a total of 1.5 to 3.0 Kg. of body weight before attaining acid base equilibrium. Usually some three-quarters of the fluid is withdrawn from the extracellular compartment and one-quarter from the cellular, although in the experiment described in Figure 26, losses were nearly equally distributed between the two compartments.

Net losses of sodium over a 5 day period have varied from 170 to over 300 mEq. in normal subjects. Potassium losses have varied from one third to half or more of sodium losses. The repair of depleted body reserves of sodium and potassium is illustrated in the last 6 days of the experiment shown in Figure 26. The excretion of cations decreased to very low levels during the first two days of recovery, although dietary intake remained the same. Anions were eliminated largely in combination with ammonia, while sodium and potassium were retained to replenish depleted extracellular and cellular reserves.

*Mechanism of the Diuresis Induced by Acidifying Salts.* The factors outlined above constitute the basis for an explanation of the diuretic action of ammonium chloride. The hydrochloric acid formed in the liver is in part neutralized by bicarbonate of blood and interstitial fluid. Bicarbonate is converted to chloride and the carbon dioxide is expelled by the lungs. In the experiment described in Figure 26 plasma chloride increased from 110 to 123 mEq per liter, plasma bicarbonate decreased from 27 to 15 mEq. per liter. The sum of the two anions remained essentially constant. The load of chloride delivered into the tubules in the glomerular filtrate increased as a result of the increase in plasma concentration. The filtered load of bicarbonate decreased. Within limits the capacities of the tubules to reabsorb chloride and bicarbonate vary reciprocally, but the reciprocity is not perfect. Although chloride reabsorption increased, some of that which was filtered escaped into the urine. On the first day sodium was sacrificed to neutralize urinary chloride. On the second and third days increasing quantities of potassium were excreted in exchange for sodium, thereby protecting sodium reserves from further depletion. From the first day onward, ammonia was substituted for sodium in steadily in-

though severely depleted of sodium. The glutaminase activity of their renal tubules is probably high. Accordingly, when ammonium chloride is administered, the chloride is largely excreted in combination with ammonia and potassium; little sodium is lost. No doubt low glomerular filtration rate and excessive salt retaining hormone production contribute to failure of diuresis in edematous patients actively conserving sodium.

*Dose and Route of Administration.* Ammonium chloride is administered orally in doses of 2 to 5 gm., 3 times a day for not longer than 4 days. Three days to a week are allowed to elapse between courses. When given to potentiate mercurial diuresis, the drug is given for 3 to 4 days, and on the last day, 2 ml. of a mercurial diuretic is given intramuscularly. Although it is commonly done, ammonium chloride should not be given as enteric coated tablets. When so administered, absorption is completely unpredictable, and usually inadequate. The taste of ammonium chloride is unpleasant and difficult to mask; syrup of raspberry is probably the most effective disguise. The drug should not be administered in a solution stronger than 2.5 per cent because of gastric irritation. It can be given in uncoated or gelatin coated tablets or in gelatin capsules along with ample water. It should be taken immediately before meals to minimize gastric discomfort. Ammonium chloride can be given intravenously in 1 to 2 per cent solution, made up in 5 per cent glucose. It must be given very slowly to avoid severe toxic reactions, at a rate not in excess of 2 gm. per hr. Under no circumstance should it be administered in such fashion to a patient with cirrhosis or evidence of hepatic dysfunction. Intravenous ammonium chloride is usually employed only in the treatment of severe metabolic alkalosis with vomiting, not for its diuretic effect.

*Toxicity.* Symptoms of gastrointestinal irritation are common, including epigastric distress, anorexia, nausea and vomiting. Symptoms of acidosis, mild in character, are to be expected if the dose of ammonium chloride is adequate. These include barely noticeable hyperventilation at rest but definite exertional dyspnea. Mild weakness and lassitude are common complaints. However, in the presence of severely impaired renal function, a fulminating type of

observed that the infusion of neutral sodium sulfate in normal subjects increases the acidity of the urine and the output of ammonia. When their subjects were depleted of sodium over a period of several days and sodium sulfate was then infused, the urine became intensely acid and far greater quantities of ammonia were eliminated. Presumably sodium depletion per se caused an adaptive increase in enzyme activity for no apparent change in acid base balance occurred as a result of sodium depletion. Enhanced exchange of hydrogen for sodium and greater production of ammonia permitted salvage of more sodium and a more rapid restoration of depleted sodium stores. However, intracellular acidity may well be a significant factor under other circumstances.

*Clinical Use of Ammonium Chloride.* Ammonium chloride is generally regarded as a mild diuretic in its own right. As pointed out above, therapeutic amounts produce a weight loss of 1.5 to 3.0 Kg. in normal subjects. In patients who respond favorably, the diuresis may be far more significant because of the availability of greater reserves of extracellular fluid. Ammonium chloride is a significant element of the Schemm regimen for the treatment of edema, which includes salt restriction, acidification, and the forcing of fluids. It plays an adjuvant role in diuretic therapy with osmotic diuretics and with chlorothiazide; in the latter instance it corrects the mild alkalosis induced by the primary diuretic drug. It is most widely used for its highly significant potentiation of mercurial diuresis (*cf.* Chapter XVI), and to correct the alkalosis which commonly results from intensive therapy with mercurial diuretics.

Ammonium chloride is perhaps ideally suited as a primary diuretic to overcome the slight tendency for fluid and salt retention which so discourages patients on a low caloric weight reducing regimen. The fact that it is apt to produce some nausea is an asset under these circumstances.

Frankly edematous patients, actively retaining sodium, rarely respond in satisfactory fashion to ammonium chloride alone, or else exhibit diuresis on the first day and become refractory thereafter. The findings of Schwartz *et al.*, alluded to above, explain this failure to respond. Such patients, although edematous, behave as

proper fashion, these two agents are the most powerful diuretics available to the physician today.

## REFERENCES


1. PITTS, R. F.: Renal excretion of acid. *Federation Proc.*, 7:418, 1948.
2. PITTS, R. F.: Acid base regulation by the kidneys. *Am. J. Med.*, *9:* 356, 1950.
3. PITTS, R. F. Modern concepts of acid base regulation. *A.M.A. Arch. Int. Med.*, *89*:864, 1952.
4. PITTS, R. F.: Mechanisms for stabilizing the alkaline reserves of the body. *Harvey Lect.*, *48*:172, 1952-53.
5. SARTORIUS, O. W , ROEMMELT, J. C., and PITTS, R. F.· The renal regulation of acid base balance in man. IV. The nature of the renal compensations in ammonium chloride acidosis. *J. Clin. Invest.*, *28*:423, 1949.

acidosis develops with Kussmaul breathing, prostration, confusion, coma, azotemia and oliguria. When patients with moderate impairment of renal function are maintained on daily doses of ammonium chloride over long periods of time, such a picture may develop gradually. Since, as pointed out above, continuous therapy with ammonium chloride serves no useful purpose; a regimen which results in severe chronic acidosis cannot be too heartily condemned. Depletion of body stores of postassium may likewise result from prolonged administration of ammonium chloride, especially if anorexia prevents adequate dietary replacement. Dietary supplements of potassium are advisable. Signs of ammonia toxicity, including weakness, apathy, drowsiness, confusion and a coarse flapping tremor may develop in cirrhotic patients with hepatic insufficiency.

*Contraindications.* Ammonium chloride should not be given to patients with severely impaired renal function because of the danger of severe acidosis. The drug should not be given to patients with evidence of liver insufficiency because of the danger of ammonia toxicity and liver coma.

## SUMMARY

Ammonium chloride induces hyperchloremic metabolic acidosis. Because plasma concentration increases, the filtered load of chloride delivered into the renal tubules increases. Although tubular reabsorption is enhanced, the excess load of chloride is not entirely removed from the urine. The chloride excreted on the first day is neutralized almost entirely by sodium; on the second and third days, it is neutralized to a significant extent by potassium. By the fifth day urinary chloride is almost entirely neutralized by ammonia. Loss of sodium during the first few days is accompanied by loss of extracellular water; loss of potassium, by loss of cell water. Because the diuretic response is brief, ammonium chloride should be administered in interrupted courses of short duration, separated by drug free intervals. Ammonium chloride is at best a mild diuretic and is usually employed as an adjuvant in therapy with more potent compounds. It serves its most useful purpose in potentiating the action of mercurial diuretics. Used together in

cortical secretion of aldosterone or the competitive inhibition of renal salt retention by the administration of a steroid which, although it had no salt retaining properties itself, would bind to renal tubular receptor sites and displace aldosterone. Amphenone B is a compound having the first action, namely inhibition of aldosterone secretion. Compounds designated as SC5233 and SC8109 are examples of the second type which apparently displace aldosterone competitively from the renal tubular receptor sites Just how ACTH, cortisone, prednisone, etc. exert their favorable actions is less certain at the moment.

*Indications for Steroid Therapy of Edema.* Widespread clinical experience justifies the statement that one or another of the following, ACTH, cortisone, prednisone or prednisolone, is the therapeutic agent of choice in the juvenile form of socalled "genuine lipoid nephrosis" and in the nephrotic stage of glomerulonephritis if renal function is adequate. Unfortunately, steroid therapy of nephrosis is not always successful. and other diuretic procedures have their place. Prednisone and prednisolone would seem at least theoretically preferable to ACTH and cortisone because of lesser salt retaining activity. The use of these agents in cardiac and cirrhotic edema and indeed in the nephrotic syndrome as well should be restricted to those patients who can be kept under close surveillance, maintained on a rigidly low salt intake, and observed continuously for complications of steroid therapy. Recent reports suggest that these steroids are especially useful in facilitating the elimination of water in the condition of hyponatremia which not infrequently develops following paracentesis or vigorous diuretic therapy with mercurial compounds. They also have been described as potentiating the action of acetazoleamide and mercurial diuretics in patients resistant to those agents.

Amphenone is too toxic for general therapeutic use but is especially interesting because of the light it has shed on the role of hypersecretion of steroids in primary diseases of the adrenal as well as in edema It may well be the harbinger of diuretic agents of the future. The compounds SC5233 and SC8109 are experimental drugs, do not seem especially potent, but are promising leads in a search for a new approach to diuretic therapy.

*Chapter XII*

# STEROID AND ANTISTEROID THERAPY

ACTH and cortisone were first used in the treatment of nephrosis by Farnsworth and by Luetscher just a decade ago. Some 5 years later Schemm, Camara, Heidorn, Cattan and Vesin, and others reported the surprising observation that these agents, known under most circumstances to cause sodium retention, frequently induced diuresis in patients with cardiac and cirrhotic edema as well as in those with the nephrotic syndrome. More recently two synthetic steroids, prednisone and prednisolone, related respectively to cortisone and to hydrocortisone, but having less sodium-retaining potentialities, have been employed in the treatment of a variety of edematous states by Muller, Mach, Landan, Riemer, and others.

The fact that the urinary excretion of salt retaining steroid, identified in more recent studies as aldosterone, is increased in patients with nephrotic, preeclamptic and cardiac edema and in those with cirrhosis and ascites has been demonstrated by Deming, Luetscher, Chart, Singer, Venning, and others. The additional observation that the sodium content of the urine, feces, sweat and saliva is reduced in edematous patients suggests that the concentration of aldosterone in the body fluids is high, due either to increased production or decreased destruction, and that overabundance of this steroid is a significant factor in the pathogenesis of edema and ascites. Davis has shown in the dog with experimental ascites and Marson and Werk et al have shown in patients with cirrhosis and ascites that bilateral adrenalectomy reduces sodium and water retention and peritoneal transudation. The difficulties of hormonal control of bilaterally adrenalectomized patients makes this something less than an ideal therapeutic procedure. A more rational type of treatment would be the inhibition of adrenal

hormone synthesis in the adrenal cortex, not by altering its rate of metabolism. While most of the above findings demonstrate interference with synthesis of glucocorticoids, it is evident from Rosenfeld and Bascom's observations that certain synthetic operations basic to production of aldosterone are also reversibly inhibited.

Renold et al observed that the exhibition of amphenone to a patient with a metastasizing adrenal carcinoma with Cushing's syndrome led to a cessation of urinary excretion of aldosterone and to a brisk diuresis of salt and water. McCullagh and Tretbar administered as much as 9.25 gm. of amphenone per day to patients with Cushing's syndrome with minimal signs of toxicity. Others, however, have noted that effective doses cause gastrointestinal disturbances, including nausea, vomiting, diarrhea and abdominal distension, methemoglobinemia, bone marrow depression, cutaneous eruptions and thyroid enlargement. In view of the multiplicity and potentially serious nature of the complications of therapy, amphenone does not seem to be a satisfactory drug for general clinical use.

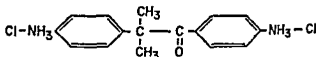
## NATURAL AND SYNTHETIC CORTICOSTEROIDS

*General Nature and Properties of Adrenal Steroids.* Approximately 30 steroids have been isolated from the adrenal cortex, most no doubt are either chemical intermediates in the processes by which the gland synthesizes its relatively few secretory products or artifacts formed in the process of chemical extraction. Intensive study of the chemistry of these compounds was begun about 25 years ago by Kendall, Pfiffner, Reichstein, Wintersteiner, and their respective associates. Their studies, which led through separation, identification of structure, to synthesis, constitute a chemical advance of tremendous significance to biology and medicine.

Figure 27 illustrates the structure of 8 steroids of significance to our discussion. All are related to allopregnane, the structure of which is shown with the several carbon atoms numbered. Those steroids with adrenal cortical biological activity of one sort or another have the following structural characteristics: (1) a double bond between carbons 4 and 5, i.e. unsaturation of ring A; (2) a

## ALDOSTERONE ANTISECRETORY AGENTS-AMPHENONE

Amphenone (1,2-bis(p-aminophenyl) - 2 - methyl propanone - 1 dihydrochloride) is one of a series of desoxybenzoins synthesized by Allen and Corwin in 1950, and has the following structure.




It was observed by Hertz and his associates to produce a marked enlargement of the adrenals of rats, associated with increased deposition of lipid materials. To a lesser extent it causes enlargement of the thyroid. In the hypophysectomized animal, neither the adrenals nor the thyroid are enlarged by amphenone. Furthermore, in the normal rat, cortisone prevents enlargement of the adrenals, and thyroid feeding prevents the development of goitre when amphenone is given.

One may infer from these facts that enlargement of the adrenals is due to enhanced ACTH secretion, and that goitre is due to enhanced TSH (thyroid stimulating hormone) secretion. The trophic hormones of the hypophysis are secreted in increased amounts because the production of adrenal steroids and thyroid hormone is suppressed. The normal feedback mechanisms, by which cortical and thyroid hormones suppress the output of trophic hormones by the hypophysis, is lost; ACTH and TSH are therefore secreted in increased amounts, leading to enlargement of the adrenals and to goitre. Hume and Nelson observed that the administration of ACTH to acutely hypophysectomized animals greatly increases the production of adrenal steroids, an action which is suppressed by amphenone. Dorfman noted that the production of 17-hydroxy corticoids in vitro by adrenal slices is inhibited by amphenone, and Rosenfeld and Bascom observed that the drug interferes with the synthesis of hydrocortisone, cortisone and corticosterone by the surviving perfused adrenal gland. Finally, Peterson, Hertz, and Lubs, utilizing isotopically tagged hydrocortisone and cortisone, have shown that amphenone exerts its effect in man by suppressing

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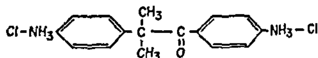
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Aldosterone was isolated in 1953 from the amorphous fraction of the adrenal cortex and its structure was determined in 1954 by Simpson, Tait, Wettstein, Euw, and Reichstein. The steroid was synthesized in 1955 by Wettstein and colleagues. It can be described chemically as 18-aldehydocorticosterone and exists in the body as an equilibrium mixture of the aldehydo and hemiacetal forms, largely the latter. Most of the sodium retaining hormone activity present in adrenal venous blood is due to aldosterone, a compound variously described as 25 to 30 times as potent as desoxycorticosterone in stimulating sodium reabsorption and potassium secretion. As was pointed out in Chapter V, its rate of secretion is relatively independent of hypophyseal trophic hormones and is possibly under the control of a hypothalamic humoral regulatory mechanism. This mechanism is activated in response to a diminution in extracellular volume or some derivative of volume, such as pressure or flow.

Aldosterone is metabolized by the liver as are so many steroid hormones. A minute fraction, probably not more than 1 or 2 per cent of the total output of the glands, is excreted in the urine. Most of the urinary hormone is excreted in a conjugated form, having no biological activity, but hydrolyzable at pH 1.0 to the parent active compound The normal individual on a liberal salt intake excretes from 1 to 4 $\mu$gm. per day; the cardiac in severe congestive failure may excrete upwards of 50 $\mu$gm. per day; and the decompensated cirrhotic, actively accumulating ascites, may excrete from 50 to 300 or more $\mu$gm. per day. While there is little doubt that aldosterone secretion is greatly increased under conditions of active salt retention, there is no way to partion increased urinary excretion between increased production and decreased tissue and hepatic destruction. From the results of Yates et al one may reasonably infer that the cirrhotic or congested liver metabolizes the hormone less effectively than does the normal.

## COMPETITIVE INHIBITION OF MINERALOCORTICOID ACTIVITY

Two synthetic steroids, designated respectively as SC5233 and SC8109, exhibit the intriguing property of reversibly inhibiting

carbonyl oxygen on $C_3$; (3) a 2 carbon side chain attached to $C_{17}$ of ring D with a ketone oxygen on $C_{20}$ of this side chain and a hydroxyl group on $C_{21}$ (α-ketol side chain). (4) Compounds with oxygen atoms on both $C_{11}$ and $C_{17}$ such as cortisone, hydrocortisone, and the synthetic analogues, prednisone and prednisolone

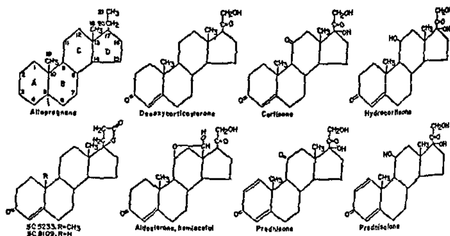



Fig. 27. Structure of natural and synthetic steroids which have an effect on the renal tubular reabsorption and excretion of ions

have predominantly glucocorticoid activity with lesser effects on electrolyte metabolism. Aldosterone is an obvious exception to this rule. (5) Further unsaturation of ring A by a double bond between carbons 1 and 2, as in prednisone and prednisolone, reduces even more the effects of these steroids on electrolyte metabolism.

*Mineralocorticoids of the Adrenal Gland.* Desoxycorticosterone was first synthesized by Steiger and Reichstein in 1937, prior to its isolation from the gland a year later by Reichstein and Euw. It is present in the adrenal cortex in minute amounts and for a time was considered to be strictly an intermediate in the synthesis of other steroids. However, it has recently been identified in adrenal venous blood and must be considered a normal glandular product. This steroid is almost devoid of glucocorticoid activity but, as was shown by Loeb and others some 20 years ago, has a profound effect on electrolyte metabolism, promoting the renal reabsorption of sodium and the secretion of potassium.

caused loss of sodium, retention of potassium, decrease in plasma bicarbonate and restoration of a normal electrocardiographic pattern. It is especially significant that these changes occurred in the presence of increased urinary excretion of aldosterone. Pre-drug excretion of aldosterone averaged 44 μgm. per day, during drug therapy, 89 μgm. per day, and on cessation of drug treatment, 44 μgm. per day. These findings rule out any feedback action whereby SC8109 might inhibit the hypothalamic controlling center and reduce secretion of aldosterone; they likewise rule out any interference with production of or any stimulation of destruction of aldosterone as an explanation of activity of the synthetic steroid.

## USE OF NATURAL AND SYNTHETIC CORTICOSTEROIDS IN THE TREATMENT OF EDEMA

*ACTH and Cortisone in Nephrotic Edema.* The nephrotic syndrome is characterized by proteinuria, hypoproteinemia, elevation of blood lipids and variable edema The form of the disease most common in childhood, namely that which has an insidious onset, without anticedent hematuria and without associated azotemia and hypertension, runs a highly variable course. Without specific therapy, the disease is marked by chronic fluctuating edema, frequently resistant to conventional diuretic therapy. Before the advent of antibiotics one in three died of infections, to which they are peculiarly susceptible, another recovered spontaneously and completely, and the third progressed to chronic nephritis and died in uremia Now that control of infection is possible, the prognosis is somewhat brighter, and as Luetscher points out, treatment should be primarily directed to survival. Attention should be directed to prevention and control of intercurrent infection, to maintenance of nutrition, to prevention of cardiac failure and to control of renal insufficiency and massive edema. Salt free concentrated serum albumin or hyperoncotic dextran administered intravenously (see Chapter VIII) and/or ACTH and adrenal steroids are useful in the control of edema, although there is no clear-cut evidence that they alter the ultimate course of the disease.

*Rationale of Use of Steroids in the Nephrotic State.* A number of clinicians have noted the association of recovery from a variety

the increased tubular reabsorption of sodium and increased secretion of potassium induced by desoxycorticosterone and by aldosterone. The structure of these steroids is shown in Figure. 27. They differ from the usual adrenal steroids in having a propionic acid lactone ring attached to the C17 site, in place of the customary $\alpha$-ketol side chain and hydroxyl group. Like desoxycorticosterone these steroids are 11-desoxy compounds. SC8109 differs from SC5233 in having a hydrogen instead of a methyl group (C19) attached to $C_{10}$. Both are known as spirolactones.

Kagawa and his associates have shown that these synthetic steroids have little effect on the excretion of sodium and potassium in the normal rat. Aldosterone, in contrast, in an amount of 0.96 $\mu$gm. markedly enhances sodium reabsorption and potassium secretion. However, the simultaneous administration of 1.2 to 1.3 mg. of SC5233 or SC8109 blocks the action of aldosterone. These steroids likewise block the renal tubular actions of desoxycorticosterone. Kagawa maintains that aldosterone and desoxycorticosterone compete with the synthetic steroids for common receptor sites on tubular cells, that this competition is reversible, and that it can be described in terms of the mass law, the affinity of the natural steroids for the receptor sites being many times that of the synthetic ones.

Liddle has carried these observations over to man, showing that SC5233 has no effect on sodium excretion in normal man on high salt intake, nor in the untreated Addisonian patient. When the patient with Addison's disease is controlled with desoxycorticosterone, SC5233 is natriuretic. Furthermore, the steroid is natriuretic in normal individuals on a low salt intake, under which condition aldosterone production is relatively great. When given to patients in congestive failure, SC5233 produces a modest diuresis of salt and water and a loss of body weight.

Salassa, Mattox and Power have confirmed the basic elements of these views in a study on a patient with primary hyperaldosteronism due to a cortical adenoma (proven at subsequent operation). The patient presented with the typical picture of polyuria, alkalosis, severe hypertension and electrocardiographic evidence of hypokalemia. The administration of 1.9 gm. per day of SC8109

and more prolonged therapy. Some patients who do not respond to steroids either during or after therapy may exhibit diuresis when treated with concentrated salt poor albumin or when given diuretics during a course of hormone treatment.

*Mechanism of Diuretic Action of Steroids in Nephrosis.* All investigators agree that accompanying or preceding loss of edema, there occurs a significant increase in glomerular filtration rate; an increase in serum sodium concentration, a marked decrease in protein excretion, an increase in plasma protein concentration and a decrease in the rate of urinary excretion of salt retaining steroids. Just which of these factors are causes and which are results of diuresis is by no means clear. There exists, according to Gaunt, an antagonism between antidiuretic hormone and the 11, 17-oxysteroids which finds its negative expression in the very slow rate of excretion of a water load by the adrenalectomized animal or by the patient with Addison's disease. The water diuresis which commonly precedes the sodium diuresis in the nephrotic patient under steroid treatment and which is no doubt responsible for the increase in serum sodium concentration, possibly results from the antagonism of the water retaining properties of ADH by the polyuric action of 11, 17-oxysteroids. In terms of the hypothesis of Wirz and Sawyer based on Ussing's work, ADH dilates pores in the distal tubules and collecting ducts, permitting the osmotic return of water to the blood stream and the formation of a small volume of concentrated urine (see Chapters IV and V); in contrast, 11, 17 oxysteroids constrict the pores, preventing reabsorption of water and leading to the formation of dilute urine.

Lauson et al have shown that ACTH abruptly decreases protein excretion due to a reduction in the abnormally high permeability of the glomerular capillaries to albumin. Associated with diminished protein excretion and elimination of excess water, serum protein concentration rises, a factor which promotes further transfer of fluid from the interstitial to the vascular compartments. What may be another manifestation of reparative processes is increased glomerular filtration rate. Eder et al logically point out that an increase in the filtered load of sodium and water serves to correct the tubular preponderance which led initially to edema formation.

of acute infections and remission of the nephrotic state. Measles has been most frequently described as effective and, in the past, some have advocated induction of this disease as a therapeutic measure. Typhoid vaccine and other forms of fever therapy have also been used to induce remission. The stress of acute infections associated with high fever probably calls forth an intense adrenal response. Increased secretion of adrenal steroids might well be the link between infectious disease and remission. Many believe that the initial renal insult in nephrosis is immunogenic in nature. The dramatic effects of ACTH and cortisone in suppressing antigen-antibody reactions suggests that this action may underlie the favorable response to adrenal steroids. No doubt curiosity as to the possible effects of new and dramatically active agents on a disease of unknown etiology must have played some role in the initial trials of ACTH and cortisone in nephrosis by Farnsworth and Luetscher. Many, including Thorn, Barnett, Lauson, Riley, and others have confirmed the fact that diuresis and remission of the disease frequently occurs during or within a day or two after cessation of treatment with ACTH and cortisone.

*Nature of the Diuretic Response in Nephrosis.* The early studies of Luetscher and Thorn indicated that ACTH was more effective than cortisone in inducing diuresis and remission of disease. Subsequently others have claimed that cortisone and prednisone are equally effective. In any event when any one of the three hormones is given in adequate dosage over a period of 10 to14 days, one of three results may be expected. (1) Diuresis begins on the second to the sixth day, initially as a water diuresis and then associated with increasing serum sodium concentration, it continues as a sodium diuresis. When therapy is stopped, a more intense diuresis occurs, resulting in complete loss of edema. (2) No diuresis occurs during steroid therapy, instead begins when hormone administration is terminated. (3) No clinical improvement results during or after therapy. Remissions vary in length from a few days to many months; indeed all evidence of disease disappears in some. In the event of exacerbation of the nephrotic state, patients are responsive to subsequent courses of therapy. Luetscher advises under these circumstances higher steroid dosage

and more prolonged therapy. Some patients who do not respond to steroids either during or after therapy may exhibit diuresis when treated with concentrated salt poor albumin or when given diuretics during a course of hormone treatment.

*Mechanism of Diuretic Action of Steroids in Nephrosis.* All investigators agree that accompanying or preceding loss of edema, there occurs a significant increase in glomerular filtration rate; an increase in serum sodium concentration, a marked decrease in protein excretion, an increase in plasma protein concentration and a decrease in the rate of urinary excretion of salt retaining steroids. Just which of these factors are causes and which are results of diuresis is by no means clear. There exists, according to Gaunt, an antagonism between antidiuretic hormone and the 11, 17-oxysteroids which finds its negative expression in the very slow rate of excretion of a water load by the adrenalectomized animal or by the patient with Addison's disease. The water diuresis which commonly precedes the sodium diuresis in the nephrotic patient under steroid treatment and which is no doubt responsible for the increase in serum sodium concentration, possibly results from the antagonism of the water retaining properties of ADH by the polyuric action of 11, 17-oxysteroids In terms of the hypothesis of Wirz and Sawyer based on Ussing's work, ADH dilates pores in the distal tubules and collecting ducts, permitting the osmotic return of water to the blood stream and the formation of a small volume of concentrated urine (see Chapters IV and V); in contrast, 11, 17 oxysteroids constrict the pores, preventing reabsorption of water and leading to the formation of dilute urine.

Lauson et al have shown that ACTH abruptly decreases protein excretion due to a reduction in the abnormally high permeability of the glomerular capillaries to albumin. Associated with diminished protein excretion and elimination of excess water, serum protein concentration rises, a factor which promotes further transfer of fluid from the interstitial to the vascular compartments. What may be another manifestation of reparative processes is increased glomerular filtration rate. Eder et al logically point out that an increase in the filtered load of sodium and water serves to correct the tubular preponderance which led initially to edema formation.

Filtration rate has been observed to increase as much as 150 per cent coincident with diuresis. According to Barnett et al, repeated courses of therapy may progressively increase glomerular filtration rate to or toward normal, even in patients with long standing disease and marked functional impairment. Tubular secretory capacity for para-aminohippurate and tubular reabsorptive capacity for glucose likewise improve under steroid therapy.

Early studies of Forsham and of Thorn suggest an antagonism between 11, 17-oxysteroids and desoxycorticosterone-like salt retaining hormones. Were this antagonism exerted at the target organ, the renal tubular cell, it would explain natriuresis during therapy with either cortisone or ACTH. However, it would not explain the reduced renal excretion of salt retaining steroids observed by Luetscher and others to accompany effective diuresis. If one accepts the fact that a fraction of aldosterone secretion is under pituitary control, then cortisone but not ACTH therapy would be expected to depress its secretion (i.e., feedback depression). Renal tubular fatigue, reduced sensitivity of the tubules to salt retaining hormone, and depression of secretion of aldosterone by cortisone and ACTH have all been suggested as possible explanations of depressed tubular reabsorption of sodium. If, as Farrell maintains, aldosterone secretion is controlled by a hormone produced in the hypothalamus, cortisone or similar steroids, secreted in response to ACTH, might depress the formation of the regulatory neurohumor.

*Dose and Route of Administration.* From 12.5 to 25 mg. of ACTH is given intramuscularly every 6 hr. for a total daily dose of 50 to 100 mg. Cortisone or prednisone is given orally in four equal doses at 6 hr. intervals; the total daily dose of cortisone is 200 mg; that of prednisone, 50 mg. Treatment is continued for 10 to 14 days and the drug is then abruptly withdrawn. If diuresis does not result either during or on cessation of therapy, the course may be repeated and after a few days of treatment, acetazoleamide, a mercurial diuretic, or no doubt other diuretics as well may be given. The patient ordinarily refractory to such diuretics, may respond well while on steroid therapy. During steroid therapy, dietary sodium intake must be rigidly restricted to between 10 and

30 mEq. per day (0.5 to 1.5 gm. NaCl per day) to avoid excessive fluid retention, and the possible complications of hypertension, congestive failure, pulmonary edema and convulsions.

*Effects of ACTH, Cortisone, Prednisone, etc., in Other Edematous States.* It is difficult to conceive that administration of steroids which are at least modestly salt retaining could exert a favorable effect on fluid and electrolyte balance in patients with congestive failure, with cirrhosis and ascites, or with pre-eclampsia. Indeed this conceptual hurdle probably accounts for the fact that steroid therapy of edema in these diseases was delayed some 5 years after its introduction in the treatment of nephrosis. In retrospect, one can provide a rationale for the use of corticosteroids on the basis of promotion of water diuresis by 11, 17 oxysteroids and a possible antagonism of the renal tubular effects of mineralocorticoids by glucocorticoids suggested by Thorn, Jailer and others.

ACTH was first used by Schemm and Camara in 1954 to treat mercury resistant, hyponatremic patients in severe congestive failure. Their regimen includes strict dietary salt restriction (9 to 30 mEq. per day), an acid ash diet plus 1.5 to 4.0 gm. of ammonium chloride per day, a bountiful fluid intake (2500 to 4000 ml. per day), adequate digitalization, bed rest, sedation, oxygen if needed, and ACTH, 15 to 25 mg. q. 6 hr. Frequently on the third to the sixth day profuse water diuresis occurs, followed by salt diuresis and loss of weight. If no diuresis occurs by the sixth day, 2 ml. of Thiomerin is given intramuscularly. Often a profound diuresis and loss of weight ensue in patients previously unresponsive to diuretic therapy. Camara and Schemm maintain that an adequate diuretic response can be obtained in 80 per cent of patients with hopeless, terminal, mercury resistant congestive failure.

More recently Cattan and Vesin, Riemer, Muller, Fabre and others have administered prednisone and prednisolone, steroids with only minor salt retaining potentialities to patients with cirrhosis and ascites, congestive failure, pre-eclampsia and nephrosis. The response has been observed to be favorable in a high proportion of cases and essentially similar to that described above, namely (1) diuresis during therapy, (2) diuresis on discontinua-

tion of therapy, (3) return of sensitivity to diuretic therapy previously resistant patients. The response that has been mo frequently observed has been increased sensitivity to mercuria and carbonic anhydrase inhibitors.

*The Mechanism of Action of Steroids in Edemas Other Tha Nephrotic* is mysterious to say the least. Indeed the very existenc of diuretic activity in patients with congestive failure or cirrhos with ascites renders suspect certain of the theories advanced abov in explanation of diuretic action of steroids in nephrosis. There i no dearth of hypotheses, yet no one of them is adequately sup ported by experimental fact. The following constitute a repre sentative sample: (1) antagonism of water retention due to ADH by adrenal corticoids, an action considered to be of special sig nificance in the correction of hyponatremia; (2) increase in glom erular filtration rate, favoring spontaneous diuresis and/or a return of sensitivity to diuretics; (3) inhibition of aldosterone production (4) increase in vasomotion of the terminal vascular bed, favoring return of fluid from the interstitial to the vascular compartments, (5) acceleration of activity of the tissue spreading factor which again favors return of fluid to the circulation, (6) decrease of permeability of glomerular and hepatic capillaries to protein; (7) an alteration of the "set" of the volume regulatory mechanism; (8) antagonism at the target organ (kidney) of mineralocorticoids by glucocorticoids.

A number of investigators have observed an increase in glomerular filtration rate in edematous patients during steroid therapy. While, to the author, it seems evident that any increase in filtration will tend to reduce the glomerulo-tubular imbalance which causes fluid retention, it is interesting to see how frequently and vociferously this is denied. Those who have measured aldosterone excretion have found it decreased under steroid therapy. Muller, however, has added the confusing but significant point that when Diamox or mercurials alone are administered to edematous patients, diuresis is accompanied by increased aldosterone excretion. When these same drugs are given to patients under prednisone therapy, diuresis is accompanied by decreased aldosterone secretion. While increased aldosterone secretion in response to potent diuretics is

easy to explain as a compensatory response to reduction of extracellular volume in a patient with an unaltered basic drive to retain salt, it is difficult to account for the opposite response with combined steroid and diuretic therapy. It implies that steroids correct the basic abnormality of congestive failure or cirrhosis (hard to believe), or that they alter the "set" of the volume control center (no evidence).

*Dose and Route of Administration* are the same in cardiac, cirrhotic and preeclamptic edema as in nephrotic edema described above. The major beneficial result is frequently increased sensitivity to more conventional diuretic agents in patients who previously responded poorly.

*Complications of Steroid Therapy.* One of the major hazards of steroid therapy is excessive retention of salt and water and precipitation of pulmonary edema. This may be avoided by strict limitation of salt intake (ideally to 10 mEq. of sodium per day), provision of an acid ash diet and administration of ammonium chloride. Despite precautionary measures some gain in weight can be expected initially. Hypertension is a complication, at least in part related to fluid retention. Other hazards include activation of peptic ulcer and hemorrhage from esophageal varices. For these reasons it is advisable to perform daily benzidene tests on the stool. Suppression of general systemic reaction and local tissue reaction to infection and irritation may mask serious acute infectious disease and render silent perforation of an ulcer.

## SUMMARY

ACTH, cortisone, prednisone, and prednisolone have recently been employed in the treatment of edema and ascites in congestive failure, cirrhosis and pre-eclampsia in much the same fashion and with much the same result noted in their earlier use in the nephrotic state. They may be employed in the treatment of acute dilutional hyponatremia, in which condition they induce first a water diuresis which may be followed by a sodium diuresis when the hyponatremia is corrected. They also frequently restore responsiveness to mercurial diuretics and carbonic anhydrase inhibitors in patients who have become resistant to these drugs.

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The mechanism of their diuretic action is by no means clear. It may be that they induce water diuresis by antagonizing the action of ADH on the renal tubule. They may likewise antagonize the salt retaining effects of aldosterone. They usually cause an increase in filtration rate, a factor of some significance both in their primary diuretic action and in their potentiation of other diuretics. They may well act peripherally by altering capillary permeability, the distribution of fluid between capillaries and tissue interstices and the ion equilibria between cells and interstitial fluid.

## REFERENCES

1. *An International Symposium on Aldosterone.* Edited by Muller, A. F. and O'Conner, C. M. Boston, Little, Brown & Co., 1958.
2. Fabre, J.: Le traitement des oedemes. *Helvet. med. acta, 23:*381, 1956.
3. Farrell, G.: Regulation of aldosterone secretion. *Physiol. Rev., 38:*709, 1958.
4. Gross, F.: Nebennierenrinde und Wasser-Salzstoffwechsel unter besonderer Berücksichtigung von Aldosteron. *Klin Wchnschr., 34:*929, 1956.
5. Hydrocortisone, its newer analogs and aldosterone as therapeutic agents. *Ann. New York Acad. Sc., 61:*281, 1955.
6. McCullagh, E. P. and Tretbar, H. A.: Treatment of Cushing's syndrome with amphenone: Report of two cases, one with probable thymoma. *J. Clin. Endocrinol & Metab., 18:*134, 1958.
7. Salassa, R. M., Mattox, V. R. and Power, M. H.: Effect of an aldosterone antagonist on sodium and potassium excretion in primary aldosteronism. *J. Clin. Endocrinol. & Metab., 18:*787, 1958.

*Chapter XIII*

# WATER IN DIURETIC THERAPY

THE importance of reducing sodium intake in the treatment of edema was increasingly appreciated over the first few decades of the present century. Today sodium restriction is accepted as a necessary procedure, limited mainly by the practicalities of providing a nutrious and reasonably appetizing diet. There is less general agreement concerning optimum water intake, and recommendations range from the archaic and inhumane view that fluids should be drastically restricted to the opposite extreme that fluids should be forced to the extent of 5 to 10 liters per day. Most clinicians favor a middle course, allowing moderate or ad libitum intake. Schroeder, Bridges, Crutchfield and many others have shown that restriction of sodium intake to a level below urinary output results in decrease of edema independent of the amount of water ingested. Furthermore, many have observed that digitalization, bed rest and other diuretic procedures cause weight loss when patients are taking liberal quantities of fluids but are ineffective when fluids are rigidly restricted

Sir Thomas Witherly's remarks[16] in a report before the Royal College of Physicians in 1690 are illuminating in this respect. "A Wine-Cooper fell into a Dropsy which resisted all the usual Methods. This Man was prodigiously swell'd, Belly, Back, Thighs and Legs. Being past all Hopes and having on him an inextinguishable Thirst, he was permitted to drink 14 Quarts of Water in about 10 hours, and in all that Time made not one Drop of Urine. Soon after he began to piss, and he drank on, 4 or 5 Quarts daily, and so recovered.—That Water should expel Water is a Miracle beyond any of St. Winifred's. Now no Man in his Senses would have prescribed such a water-course to cure a Dropsy, which

[16] Quoted from F. R. Schemm *Ann. Int. Med.*, *21*:937-976, 1944

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1. *An International Symposium on Aldosterone.* Edited by Muller, A. F. and O'Conner, C. M. Boston, Little, Brown & Co., 1958.
2. Fabre, J.: Le traitement des oedemes. *Helvet. med. acta, 23:*381, 1956.
3. Farrell, G.: Regulation of aldosterone secretion. *Physiol. Rev., 38:* 709, 1958.
4. Gross, F.: Nebennierenrinde und Wasser-Salzstoffwechsel unter besonderer Berucksichtigung von Aldosteron. *Klin Wchnschr., 34:*929, 1956.
5. Hydrocortisone, its newer analogs and aldosterone as therapeutic agents. *Ann. New York Acad. Sc., 61:*281, 1955.
6. McCullagh, E. P. and Tretbar, H. A.: Treatment of Cushing's syndrome with amphenone: Report of two cases, one with probable thymoma. *J. Clin. Endocrinol & Metab., 18:*134, 1958.
7. Salassa, R. M., Mattox, V. R. and Power, M. H.: Effect of an aldosterone antagonist on sodium and potassium excretion in primary aldosteronism. *J. Clin. Endocrinol. & Metab., 18:*787, 1958.

dextrose from 1 to 6 times per day. At first he observes no diuresis, a gain in weight and a visible increase in edema. Persistence is rewarded by diuresis and clearing of edema.

Vital elements of the Schemm regimen are rigid dietary sodium restriction, ideally to 9 mEq. per day; an acid ash diet supplemented with 1.5 to 4 0 gm. of ammonium chloride; bed rest; and digitalization, sedation, and oxygen for the patient in congestive failure, if needed. No doubt a number of the failures of those who have tried this regimen, have been due to inadequate restriction of sodium intake and/or inadequate attention to other elements of the regimen. However, acute dilutional hyponatremia has frequently resulted from the inadequate diuretic response of severely ill and edematous patients. Most who have tried the Schemm regimen have been less impressed with its efficacy as a primary therapeutic procedure than has he. The reason for discussing it is to emphasize once again that dehydration by fluid restriction defeats rather than promotes therapy of edema.

*Mechanism of Salt Loss in Water Diuresis.* Recent studies of Leaf, Weston, Wrong and their respective associates demonstrate clearly that salt loss can be induced in normal subjects by hyperhydration and that the magnitude of the loss is related primarily to the degree of expansion of volume of body water. They observed that subjects given daily doses of pitressin tannate in oil to induce antidiuresis and loaded judiciously with water, retain that water, develop an acute dilutional hyponatremia, expand cellular, interstitial and plasma stores of water, and after some 6 to 12 hr. exhibit a significant sodium diuresis. From 70 to 370 mEq. of sodium may be lost per day. When the administration of pitressin is stopped, water diuresis ensues over the succeeding 24 to 48 hr. and at its end, weight is reduced in proportion to the sum of the daily losses of sodium. Leaf maintains that sodium loss can be reduced by the coadministration of ACTH. In a similar vein, Wrong maintains that the delay of 6 to 12 hr. in the onset of saluresis after the imposition of a positive water load represents the time required for the relatively slow hepatic metabolism of circulating aldosterone.

shows how little we know of Nature and the great Uncertainty of our Art."

That water can expel water was observed in controlled experiments of Marshall in 1920, of Gamble in 1937, of Stewart and Rourke and of Schemm in 1942, of Wolf in 1945 and of others more recently. While the fact is certain that the forcing of fluids can, under proper circumstances, lead to dehydration, the efficacy of the procedure as a primary means of treating edema is not generally accepted.

*The Dehydrating Effects of Water.* Wolf observed that when normal subjects drink from 20 to 200 ml. of water every 10 min. for from 3 to 7 hr., urine flow increases rapidly and is sustained at a level some 8 per cent above intake. If one includes insensible water loss through lungs and skin in balance calculations, it is evident that total output of water can exceed intake by as much as 15 to 20 per cent. Wolf observed that urinary chloride concentration tended to be high at the start of the drinking period, to drop sharply over the first hour or so, and to reach a plateau of 1.2 mg. per ml. (33 mEq. per liter), after 3 hr. If urine flow were sustained for 7 hr. at the maximum rate of 20 ml. per min, the total salt lost would be equivalent to that contained in 2 liters of extracellular fluid, and at the end of diuresis, body weight would decrease 2 Kg. Two facts militate against such an ideal response in the edematous patient: diuresis is rarely as adequate as in the normal, in consequence of salt retention, the plateau of minimum urinary sodium and chloride concentration is lower. Furthermore, there is the ever present hazard of inducing acute dilutional hyponatremia of serious proportions.

*The Schemm Regimen.* Schemm has noted that while the mildly edematous patient does well on a fluid intake of 2500 to 3000 ml. per day, the severely edematous patient when first seen is occasionally seriously dehydrated in the sense that osmolality of the body fluids is increased in consequence of prolonged and ill-advised fluid restriction. For such patients Schemm recommends as much as 8 to 10,000 ml. of fluid for a day or so and 4 to 5,000 ml. per day thereafter. If such amounts of water cannot be tolerated by mouth, he recommends 500 to 1000 ml. of isotonic

fluids. However, many seriously ill patients cannot tolerate the dilution necessary to attain a significant saluretic response, if indeed it can be attained at all.

A point of some interest is the incapacity of the seriously ill edematous patient to exhibit water diuresis in response to hypotonic expansion of body fluids. Failure to respond to the ingestion of water with increased urine flow has been generally explained in terms of excessive secretion of ADH. The supraoptico-hypophyseal antidiuretic mechanism, although primarily sensitive to the osmotic pressure of the body fluids, has been considered to be subject to secondary control by the volume regulatory mechanism. This view has been questioned by Lamdin et al who have found that alcohol, which inhibits release of ADH from the neurohypophysis, does not restore water diuresis in patients with cirrhotic, nephrotic and cardiac edema. Recent experiments of del Greco, Kleeman, Berliner and their respective associates demonstrate that an acute reduction in glomerular filtration rate limits water diuresis. A sufficient reduction in filtration rate (20 to 30 per cent) causes oliguria of hypertonic urine, even though body fluids are dilute and secretion of ADH is suppressed. These facts suggest that reduction in filtration rate in the edematous patient may contribute to the reduced diuretic response to water loading. Other factors no doubt contribute. The author believes that excessive secretion of ADH may play some role in limiting water diuresis in edema, however, it cannot be the entire explanation; reduced filtration rate and other unknown factors also play significant roles.

## SUMMARY

Providing the intake of sodium is sufficiently reduced, the intake of water has relatively little effect on the rate of accumulation of edema. In normal subjects and in some edematous patients, large water loads cause the development of negative sodium balance. Water expels water and body weight is reduced. However, the seriously ill edematous patient may respond adversely to large water loads by developing hyponatremia and water intoxication without exhibiting either diuresis or saluresis. The intake of mod-

By definition, the saluresis which follows hyperhydration results from glomerulotubular imbalance due to relative glomerular preponderance. No less than three factors may play some role in its induction, although their relative importance cannot be assessed at the moment. Bartter has shown that water loading in a pitressin treated subject reduces the rate of urinary excretion of aldosterone (see Chapter V), and presumably its rate of glandular secretion as well. In consequence of reduced circulating hormone, the rate of reabsorption of salt by the renal tubule is reduced; salt excretion is increased. Hyperhydration also increases glomerular filtration rate. In the studies of Leaf, filtration rate increased from 20 to 30 per cent. More salt containing isotonic fluid is delivered from the proximal tubules into loops of Henle, distal tubules and collecting ducts. If the reabsorptive capacities of these segments of the nephron remain relatively unchanged, the greater filtered load of salt would be less completely reabsorbed and excretion would increase. If in addition, reabsorptive capacity is reduced because of reduced secretion of aldosterone, excretion would increase still more. A final factor of unknown significance is velocity of flow of tubular urine along the collecting ducts. It is possible, though by no means proven, that absorption of the final traces of sodium is rendered less complete by high rates of flow in water diuresis.

*Role of Hydration in the Therapy of Edema.* It seems reasonable to assume that extracellular fluid volume is controlled by a receptor-hypothalamic integrator-neurohumoral effector system, which determines the balance between filtered load and tubular reabsorption of sodium (see Chapter V). It is a truism to state that this mechanism operates abnormally in edematous patients. Dehydration, though it may reduce somewhat the volume of interstitial fluid, stimulates intensely mechanisms of salt retention; diuretic therapy is rendered less effective. Provision of ample water, though it expands extracellular and cellular volume, i.e., increases edema, lessens the intensity of salt conservation; diuretic therapy is rendered more effective. Hyperhydration actually promotes salt loss in those patients in whom filtration rate can be sufficiently increased and aldosterone secretion sufficiently depressed by tolerable degrees of hypotonic expansion of volume of body

fluids. However, many seriously ill patients cannot tolerate the dilution necessary to attain a significant saluretic response, if indeed it can be attained at all.

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erate quantities of water, ad libitum in the alert and responsive patient, some 2000 to 3000 ml. in the depressed or comatose patient, supplies water needs, provides for an adequate urine volume and renders the patient more responsive to diuretic therapy. Restriction of fluid intake, except immediately after paracentesis or massive diuresis, is inhumane and without effect on the ultimate accumulation of edema, for salt retention continues and the body is diluted when water restriction is relaxed. Furthermore, water restriction reduces the patients response to diuretic therapy.

## REFERENCES

1. Berliner, R. W. and Davidson, D. G.: Production of hypertonic urine in the absence of pituitary antidiuretic hormone. *J. Clin. Invest.*, *36*:1416, 1957.
2. Lamdin, E., Kleeman, C. R., Rubini, M., and Epstein, F. H.: Studies on alcohol diuresis. III. The response to ethyl alcohol in certain disease states characterized by impaired water tolerance. *J. Clin. Invest.*, *35*:386, 1956.
3. Schemm, F. R.: High fluid intake in the management of edema, especially cardiac edema: the details and basis of the regime. *Ann. Int. Med.*, *17*:952, 1942.
4. Wolf, A. V.. Dehydrating effect of continuously administered water. *Am. J. Physiol.*, *143*:567, 1945.
5. Wrong, O.. The relationship between water retention and electrolyte excretion following administration of antidiuretic hormone. *Clin. Sc.*, *15*:401, 1956.

*Chapter XIV*

# OSMOTIC DIURESIS

IN response to prolonged thirsting and fasting, the urine flow of a normal individual decreases to 0.1 to 0.3 ml. per min. and urine osmolality increases to a maximum of 1200 to 1400 mOsm. per liter, i.e., to a value some 4 to 5 times that of the plasma. Since urine osmolality is determined largely by content of urea and electrolytes, minimum urine volume is ultimately dependent on the load of these substances demanding excretion. Any increase in load must result in an increase in volume. When any excretory solute, whether a normal urinary constituent or a foreign substance, is administered in a concentration higher than that in which it can be eliminated, water is abstracted from the body. The solute serves as an osmotic diuretic. Were water alone to be abstracted from the body, no useful end would be achieved, for thirst would drive the individual to restore his water deficit. Such therapeutic benefits as derive from the use of osmotic diuretics result from the fact that sodium excretion increases more or less in proportion to the increase in urine flow.

Osmotic diuretics are not especially potent therapeutic agents and their limited clinical utility scarcely justifies an extended discussion. However, the experimental exploitation of osmotic diuretics has contributed greatly to an understanding of renal function. Furthermore, all effective diuretics in some degree induce osmotic diuresis, for by inhibiting the reabsorption of sodium and either chloride or bicarbonate ions, they increase the osmotic load of electrolytes demanding excretion. The excretion of electrolytes obligates the excretion of water, extracellular volume is reduced, and body weight declines. The reader will find that this chapter is more concerned with the functional than with the therapeutic implications of osmotic diuresis.

erate quantities of water, ad libitum in the alert and responsive patient, some 2000 to 3000 ml. in the depressed or comatose patient, supplies water needs, provides for an adequate urine volume and renders the patient more responsive to diuretic therapy. Restriction of fluid intake, except immediately after paracentesis or massive diuresis, is inhumane and without effect on the ultimate accumulation of edema, for salt retention continues and the body is diluted when water restriction is relaxed. Furthermore, water restriction reduces the patients response to diuretic therapy.

## REFERENCES

1. Berliner, R. W. and Davidson, D. G.: Production of hypertonic urine in the absence of pituitary antidiuretic hormone. *J. Clin. Invest.*, *36*:1416, 1957.
2. Lamdin, E., Kleeman, C. R., Rubini, M., and Epstein, F. H.: Studies on alcohol diuresis. III. The response to ethyl alcohol in certain disease states characterized by impaired water tolerance. *J. Clin. Invest.*, *35*:386, 1956.
3. Schemm, F. R.: High fluid intake in the management of edema, especially cardiac edema. the details and basis of the regime. *Ann. Int. Med.*, *17*:952, 1942.
4. Wolf, A. V.: Dehydrating effect of continuously administered water. *Am. J. Physiol.*, *143*:567, 1945.
5. Wrong, O.: The relationship between water retention and electrolyte excretion following administration of antidiuretic hormone. *Clin. Sc.*, *15*:401, 1956.

papillary interstitium. The osmolality of the urine approaches that of the tissue forming the tip of the papilla. In osmotic diuresis, the presence of unreabsorbable solute in the glomerular filtrate restricts the isotonic reabsorption of sodium and water in the proximal tubules. Since distal reabsorption is limited, a much larger volume of isotonic fluid is delivered into the collecting ducts. One may theorize that the reabsorption of more water at this site reduces the hypertonicity of the papillary tissue, hence reduces the final osmolal concentration of the urine, and increases the volume flow of urine. The mechanisms of increased urine flow and increased sodium excretion in osmotic diuresis will be considered in the following pages.

*Osmotic Load, Urine Osmolality, and Urine Flow* are interrelated in the manner described in Figure 28 A, B, redrawn in idealized form from data of Raporort *et al.* Figure 28A describes the relationship between urinary osmotic load and urine flow in a series of young normal subjects fasting and thirsting for 16 hrs. prior to the experiment. Urine osmotic load is defined as the rate of excretion of osmotically active solutes, namely the product of urine osmolality and urine flow ($U_{osm} \times V$). In control observations of Figure 28A, both urine osmotic load and urine flow were low. The data grouped at the lower left hand end of the curve. The urine osmotic load was increased progressively by the intravenous infusion of a hypertonic solution of one of a variety of solutes. Urine flow increased to values as high as 22.8 ml. per min. The relationship between urine flow and urine osmotic load was the same, independent of the nature of the solute, and was thus dependent solely on rate of excretion of osmotically active particles, not on their chemical constitution. The solutes included glucose, urea, mannitol, sucrose, sorbose, sorbitol, xylose, creatinine, sodium sulfate, sodium para-aminohippurate and sodium chloride. Experimentally, any one of these solutes could be employed as an osmotic diuretic, practically, one would avoid sodium salts in the treatment of edema. Urea is the only one of these eleven solutes employed therapeutically.

Figure 28B describes the relationship between urine flow and plasma and urine osmolality in the same series of experiments from

*Osmotic Work.* The formation of urine hypertonic to plasma involves the performances of osmotic work. Osmotic work is some function of the product of the urine/ plasma osmolal concentration ratio, i.e., the degree to which the urine is osmotically concentrated with respect to the plasma, and the volume of urine elaborated per unit time, i.e., the urine flow. Hervey, Rapoport, Newburgh and others have calculated minimum renal osmotic work under a variety of conditions, as though concentration of the urine were carried out as a thermodynamically reversible process. The formation of small volumes of maximally concentrated urine involves the expenditure of relatively little energy, roughly 0.6 gm. cal, per min. per 1.73$M^2$ surface area. If osmotically active excretory solutes are administered in hypertonic solution in progressively increasing quantities, urine flow increases and osmolal concentration decreases. However, osmotic work, related to the product of the volume and osmolal U/P ratio, increases some 7 fold to a limiting value of 4 0 gm. cal. per min. per 1.73 $M^2$ surface area. Under such conditions of osmotic diuresis, less work is performed per ml. of urine, but more work is performed per min. in consequence of increased flow. Obviously, the maximum urine concentration of 1400 mOsm. per liter at minimum flows, as observed in simple dehydration, cannot be assigned to a limitation of osmotic work capacity of the kidneys per se; rather it must find its explanation in some absolute restriction of the capacity of the nephron to concentrate the urine relative to the plasma.

According to the thesis of Wirz, Hargitay and Kuhn developed in Chapter IV, the osmotic work involved in the production of urine hypertonic to plasma is performed in the loop of Henle by *the pumping of sodium from ascending limb into the interstitium.* An increasing gradient of osmolal concentration is developed along the loop from the corticomedullary junction to the tip of the papilla and involves both tubular contents and interstitial fluid (see Fig. 10.)

In simple dehydration, a small volume of isotonic fluid containing all of the excretory products is delivered from distal tubules into collecting ducts. As the tubular fluid flows along the collecting ducts, water is reabsorbed by osmosis into the hypertonic

papillary interstitium. The osmolality of the urine approaches that of the tissue forming the tip of the papilla. In osmotic diuresis, the presence of unreabsorbable solute in the glomerular filtrate restricts the isotonic reabsorption of sodium and water in the proximal tubules. Since distal reabsorption is limited, a much larger volume of isotonic fluid is delivered into the collecting ducts. One may theorize that the reabsorption of more water at this site reduces the hypertonicity of the papillary tissue, hence reduces the final osmolal concentration of the urine, and increases the volume flow of urine. The mechanisms of increased urine flow and increased sodium excretion in osmotic diuresis will be considered in the following pages.

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Figure 28B describes the relationship between urine flow and plasma and urine osmolality in the same series of experiments from

which Figure 28A was derived. Urine osmolality decreased with increasing urine flow to approach plasma osmolality as an asymptote. In the control periods prior to infusion of solute, plasma osmolality averaged 295 mOsm. and urine osmolality 1400 mOsm. per liter. As a result of infusion of solute, plasma osmolality rose to

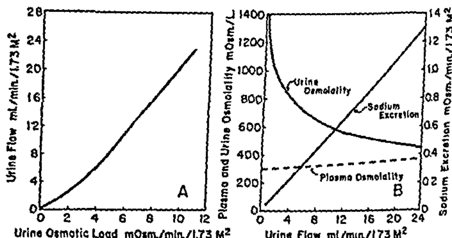



Fig. 28 Renal response to osmotic diuresis in man A. The relationship between urine flow and urine osmotic load. B. The relationship between urine osmolality, plasma osmolality, sodium excretion, and urine flow (Redrawn in somewhat idealized form from data of S. Rapoport, W.A. Brodsky, C.D. West, and B Mackler· *Am J Physiol*, *156*:433, 1949, and from data of S. Rapoport, C.D. West and W.A. Brodsky *Am. J. Physiol.*, *157*:363, 1949.)

365 mOsm. and urine osmolality decreased to 465 mOsm. per liter at the highest rate of urine flow, 22.8 ml. per min. Since the *subjects were thirsting and hydropenic at the start*, and since solutes were infused in highly hypertonic solution, the kidneys were maximally stimulated throughout the experiment by ADH to conserve water. As was pointed out above, ability to concentrate the urine is inversely related to the load of solutes demanding excretion. If solute load is small, high concentration is achieved; if solute load is large, the urine can be concentrated only to a limited extent and flow is correspondingly increased.

*The Osmolal Clearance* is defined as ml. per min. of plasma

completely cleared of osmotically active components and is calculated as follows:

$$C_{osm.} = \frac{U_{osm} \times V}{P_{osm.}}$$

where $C_{osm}$ = osmolal clearance in ml. per min , $U_{osm}$ = mOsm. per ml. of urine, $P_{osm}$ = mOsm. per ml. of plasma, and V = ml. of urine per min. Osmolalities of plasma and urine are customarily measured cryoscopically, i.e, by freezing point depression, and thus include the contributions of both electrolytes and non-electrolytes. The osmolal clearance of the normal fasting individual varies between 2 and 3 ml. per min. at urine flows of 0.3 to 0.5 ml. per min, or more.

$$C_{osm.} = \frac{1.400 \text{ mOsm./ml.} \times 0.5 \text{ ml./min.}}{0.300 \text{ mOsm./ml.}} = 2.33 \text{ ml. per min.}$$

The osmolal clearance depends largely on the rate at which urea and electrolytes are cleared from the plasma and is, therefore, constant only within rather broad limits determined by the immediately preceding dietary history.

*Osmolal Clearance and Water Conservation in Osmotic Diuresis.* If the osmolal clearance is 2.33 ml per min. and the urine flow 0.5 ml. per min., as in the example cited above, the kidneys have in essence restored to the body 2.33 − 0.5 = 1.83 ml. per min. of pure water to cover extra-renal losses or to redilute hypertonic body fluids. Water conservation is defined as the difference between osmolal clearance and urine flow ($C_{osm}$ — V.) It represents the volume of solute-free water which is reabsorbed from a volume of isotonic tubular fluid equivalent to the osmolal clearance, in order to increase its osmolality to that of the finished urine. Smith and his associates refer to water conservation as *negative free water clearance: negative,* because it represents reabsorbed water; *free water,* because it is solute-free. It is generally conceded that water conservation in the sense described above is effected in the collecting ducts.

The data presented in Table VIII are mean values derived from the curves of Figure 28 A, B. As urine osmotic load is increased from 0.7 mOsm. per min. (control) to 10 mOsm. per min. (osmotic diuresis), urine flow increases from 0.5 to 21.5 ml. per

min. and urine osmolality decreases from 1400 to 465 mOsm. per liter. Water conservation, i.e., the difference between osmolal clearance and urine flow, increases from 1.8 to 6 to 7 ml. per min., becoming roughly constant at urine osmotic loads greater than 3.0 mOsm. per min. Since water conservation in the collecting ducts is limited, the delivery of excessive volumes of isotonic fluid into these segments results in increased urine flow, i.e., osmotic diuresis.

Because of relative constancy of water conservation at high osmotic loads, Smith refers to it as $T^c_m$ $H_2O$, the maximum capacity of the concentrating segment to reabsorb water. In terms of Wirz' hypothesis, this limited capacity of the tubules to conserve water can be explained in two ways. First, $T^c_m$ $H_2O$ might be related to a limited rate at which sodium can be pumped from ascending limbs of Henle's loops into interstitium and descending limbs. If the counter current multiplier and counter current exchange mechanisms described in Chapter IV were perfectly efficient (doubtful), the pumping of an amount of sodium equal to that contained in 6 to 7 ml. of isotonic proximal fluid from ascending to decending limbs of Henle's loops could account for the osmotic transfer of an equivalent volume of solute-free water across the collecting ducts. Second, $T^c_m$ $H_2O$ might represent a constant rate of osmotic transfer of water, limited by tubular permeability to water rather than by osmotic force. The data required to formulate a definitive explanation are not available.

***Sodium and Water Excretion in Osmotic Diuresis.*** If, as pointed out above, diuresis were to cause only a loss of body water without loss of body sodium, it would serve no therapeutically useful purpose. Thirst would drive the patient to replace his water deficit and edema would reaccumulate. Actually, sodium is lost in osmotic diuresis in proportion to urine flow, a fact evident in Figure 28B. It is now generally accepted that increased sodium excretion in osmotic diuresis results from reduced reabsorption of this ion in the proximal tubules. Because less sodium is reabsorbed, less water is reabsorbed. More sodium and water are delivered into distal parts of nephrons. Because the reabsorptive capacities of these distal parts are limited, more salt and water are excreted.

TABLE VIII

RELATIONSHIPS OF OSMOLAL CLEARANCE, URINE FLOW AND WATER CONSERVATION IN OSMOTIC DIURESIS IN MAN
*(Data are mean values taken from Figure 28)*

| *Condition* | *Urine Osmotic Load* $U_{osm}.xV$ | *Urine Osmolality* $U_{osm}$ | *Plasma Osmolality* $P_{osm}$ | *Osmolal Clearance* $C_{osm}.$ | *Urine Flow* $V$ | *Water Conservation* $C_{osm}-V$ |
|---|---|---|---|---|---|---|
| | *(mOsm/min.)* | *(mOsm/ml.)* | *(mOsm/ml.)* | *(ml./min.)* | *(ml./min.)* | *(ml./min.)* |
| Control | 0.7 | 1.400 | 0.30 | 2.3 | 0.5 | 1.8 |
| Osmotic Loading | 2.0 | 0.625 | 0.30 | 6.7 | 3.2 | 3.5 |
| Osmotic Loading | 4.0 | 0.597 | 0.31 | 12.9 | 6.7 | 6.2 |
| Osmotic Loading | 6.0 | 0.545 | 0.33 | 18.2 | 11.0 | 7.2 |
| Osmotic Loading | 8.0 | 0.500 | 0.35 | 22.8 | 16.0 | 6.8 |
| Osmotic Loading | 10.0 | 0.465 | 0.36 | 27.8 | 21.5 | 6.3 |

An explanation of reduction of proximal tubular reabsorption of sodium and water in osmotic diuresis will be developed in terms of hypothetical data presented in Table IX. Under normal conditions, the glomerular filtrate is assumed to contain cations (exclusively sodium) in a concentration of 140 mOsm. per liter and anions (exclusively chloride and bicarbonate) in the same concentration. Glucose, amino acids and other valuable constituents are present in a concentration of 10 mOsm. per liter. Waste products are present in a concentration of 6 mOsm. per liter.[17] The sum of osmotically active components is 296 mOsm. per liter. The volume of filtrate is 100 ml. per min.

In the course of passage of the glomerular filtrate along the *proximal tubule, it is assumed, as in Chapter IV, that some 7/8th* of the sodium, chloride and bicarbonate and all of the valuable non-electrolyte constituents are reabsorbed. This provides the osmotic motive force to reabsorb 7/8th of the filtered water. *At the end of the proximal tubule, volume is reduced from* 100 ml. to 16.7 ml. per min., namely to 1/8th of its original value. By virtue of water reabsorption, excretory products are concentrated 8 times, from 6 to 48 mOsm. per liter. Much evidence indicates that the *proximal tubular fluid remains isosmotic with plasma; therefore,* total osmolal concentration remains unchanged at 296 mOsm. per liter. For this to be true, the concentrations of cations and anions must be slightly reduced, each to 129 mOsm. per liter. The rate of *delivery of cations and anions from the end of the proximal seg*ment into more distal parts of the nephron is 4.31 mOsm. per min. (129 mOsm./L + 129 mOsm./L) $\times$ 16.7 ml./min./1000. Under normal conditions, most of the 16.7 ml. of water and essentially all of the 4.31 mOsm. of electrolyte are reabsorbed each minute in the distal parts of the nephron.

In profound osmotic diuresis, the significant change in the composition of the plasma and glomerular filtrate is a marked increase in concentration of excretory products, in the example cited in the lower part of Table IX, to 100 mOsm. per liter. As a consequence,

[17] Waste products such as urea and uric acid are in part reabsorbed in the proximal segment. The 6 mOsm per liter represents that moiety present in the filtrate which is not reabsorbed in the proximal tubules.

the sum of osmotically active components is increased to 390 mOsm. per liter. As the filtrate flows along the proximal tubule, all valuable nonelectrolyte components are reabsorbed. However, the reabsorption of cations, anions and water is reduced. According to Wesson and Anslow, the proximal reabsorption of sodium is retarded by the development of a critical gradient between tubular urine and plasma. This critical gradient develops because excretory products in the tubular urine limit the reabsorption of

TABLE IX

HYPOTHETICAL DATA ILLUSTRATING THE PROBABLE ORIGIN OF NATRIURESIS AND ENHANCED URINE FLOW IN OSMOTIC DIURESIS

| | *Glomerular Filtrate* | | *Fluid at End of Proximal Tubule* |
|---|---|---|---|
| | *Normal* | | |
| Cations | 140 mOsm./L | | 129 mOsm./L |
| Anions | 140 mOsm./L | | 129 mOsm./L |
| Glucose, amino acid, etc. | 10 mOsm./L | | 0 mOsm./L |
| Excretory products | 6 mOsm./L | × 8 = | 48 mOsm./L |
| ΣOsmotic components | 296 mOsm./L | | 296 mOsm./L |
| Volume | 100 ml./min | × ⅛ = | 16.7 ml./min |
| ΣCations and anions at end of proximal tubule | | | 4.31 mOsm./min. |
| | *Osmotic Diuresis* | | |
| Cations | 140 mOsm./L | | 80 mOsm./L |
| Anions | 140 mOsm./L | | 80 mOsm./L |
| Glucose, amino acids, etc. | 10 mOsm./L | | 0 mOsm./L |
| Excretory products | 100 mOsm./L | × 2.3 = | 230 mOsm./L |
| ΣOsmatic components | 390 mOsm./L | | 390 mOsm./L |
| Volume | 100 ml./min. | × 1/2.3 = | 43 ml./min |
| ΣCations and anions at end of proximal tubule | | | 6.88 mOsm./min. |

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sorption of sodium. The magnitude of that gradient has not been accurately defined in the mammalian kidney.

*Characteristics of an Ideal Osmotic Diuretic* include the following. (1) It should be restricted in its distribution in the body to the extracellular fluid compartment. If, like urea, it penetrates cells, large doses must be administered. Although urea is the most commonly used osmotic diuretic, it is by no means ideal in all respects. (2) It should not be metabolized in the body. (3) It must be freely filterable through glomerular capillaries and (4) should not be reabsorbed by the renal tubules. (5) It should be readily absorbed from the gut on oral administration. (6) When administered in effective doses, it should cause no gastro-intestinal or general systemic disturbances. No osmotic diuretic has all these characteristics, and as a class, they are little used today. Urea and potassium salts have been most extensively used clinically as osmotic diuretics because they are readily absorbed from the gut and rapidly excreted in the urine. Potassium salts will be discussed in another connection in Chapter XVIII.

*Use of Urea as an Osmotic Diuretic.* Friedrich in 1892 first employed urea as a diuretic in patients with congestive heart failure and cirrhosis. Although he obtained a favorable response to doses as small as 2 to 14 gm. per day, others have found it necessary to administer from 30 to 100 gm. per day to obtain significant diuresis. In most instances from 50 to 60 gm., divided into 3 doses and administered immediately after meals, produces as great a diuresis as is likely to be obtained. The increase in daily urine output may be 2-to 4-fold in patients in whom the prediuretic output is from 300 to 700 ml. The diuretic response is roughly comparable to that induced by xanthines.

Urea is rather extensively reabsorbed by the renal tubules, i.e., some 40 to 70 per cent of that filtered is returned to the blood stream. Most investigators believe that urea is passively reabsorbed, although Schmidt-Nielsen maintains that, in part, transport is active. Shannon has demonstrated that under normal conditions as much as 40 per cent of the filtered urea may be reabsorbed in the proximal tubules, a value reduced in profound osmotic diuresis to about 10 per cent. An additional 10 to 40 per cent is reabsorbed

water and thus dilute the sodium of the tubular fluid. Failure of sodium reabsorption leaves sodium in the tubular urine, which by its own osmotic effect still further prevents reabsorption of water. This concept is illustrated in the data of Table IX. If as a result of reabsorption of sodium, glucose and amino acids, volume is reduced to 43 ml. per min., excretory products are concentrated to 230 mOsm. per liter. To meet the requirement of isotonicity of proximal urine,[14] the concentration of cations and anions must be reduced to 80 mOsm. per liter. This represents a gradient of 80/140 mOsm. per liter between tubular fluid and plasma, sufficient to prevent further reabsorption of sodium. There would be delivered into more distal parts of the nephron 43 ml. of water containing 6.88 mOsm. per min. of cations and anions (80 *mOsm./L. + 80 mOsm./L) × 43 ml./min./1000)*. If reabsorption of electrolyte and water by the distal nephron is limited to 4.31 mOsm. per min. and to some 16 ml. per min., respectively, urine flow would increase to more than 20 ml. per min. and electroylte excretion to 2.57 mOsm. per min. (6.88 — 4.31 = 2.57). The sodium moiety would be 1.28 mOsm. per min., a value of the proper order of magnitude (*cf.* Figure 28B.)

Mudge, Foulks and Gilman and more recently Thompson have claimed that no absolute gradient exists which stops sodium transfer in the proximal tubule. Rather rate of reabsorption of sodium is reduced both by a reduction in concentration of sodium and by a reduction in the length of time that the fluid is in contact with the tubular epithelium. Recently Windhager et al have shown, by perfusion experiments on the proximal tubule of Necturus, that an absolute limiting gradient exists. If the sodium concentration of the tubular perfusate is less than 2/3rds that of plasma, sodium and water enter the tubule; if more than 2/3rds, sodium and water are reabsorbed. The author, therefore, favors the explanation of Wesson and Anslow, namely that accumulation of osmotically active solute in the proximal tubule establishes a gradient between tubular fluid and plasma which prevents further reab-

[14]Both Wirz and Gottschalk have shown by micropuncture of mammalian proximal tubules that the tubular fluid is isotonic with plasma, not only under normal conditions, but also in osmotic diuresis produced by the infusion of mannitol.

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in the distal tubules, the proportion varying as an inverse function of urine flow or directly with the degree of concentration of the urine. To whatever extent urea is reabsorbed, its efficacy as an osmotic diuretic is reduced. Because of relative non-toxicity and tolerance of high blood levels, tubular reabsorption does not ordinarily limit the capacity of the kidneys to excrete osmotically significant quantities of urea in the urine. If no diuresis occurs by virtue of severely reduced renal function (low glomerular filtration rate), the blood urea concentration may rise excessively.

The excretion of sodium and chloride is increased by the administration of urea more or less in proportion to the rate of excretion of urea. The excretion of excess water and electrolyte in urea diuresis as in other types of osmotic diuresis depends on diminished proximal tubular reabsorption as described above.

Two difficulties may be experienced in the use of osmotically adequate doses of urea. (1) In some patients, gastrointestinal disturbances, including nausea and vomiting, may preclude its use. If administered immediately after meals, little disturbance is usually encountered. All patients complain of its disagreeable taste, which can be only partially masked by flavoring agents. (2) In patients with severely reduced renal function, nitrogen retention occurs, accompanied by weakness, lassitude and loss of appetite, necessitating discontinuation of the drug. In patients with marked liver insufficiency, ammonia toxicity and hepatic coma may result as a consequence of conversion of urea to ammonia in the gut.

Except in patients with severe renal or hepatic insufficiency, urea is nontoxic, even on prolonged use. Undiminished diuretic potency over a period of years and a more or less additive response when combined with other agents increase its usefulness. It is properly claimed to be the safest, but certainly not the most effective of all diuretics.

*Use of Other Solutes as Osmotic Diuretics.* A variety of organic solutes, including glucose, sucrose, xylose, mannitol, sorbitol, sorbitan, and creatinine have been employed experimentally as osmotic diuretics. All must be administered intravenously to be effective; glucose, because of rapid metabolism; sucrose, because of digestion; and the remainder, because of poor intestinal absorp-

tion and resulting purgation. For this reason they are clinically less useful than urea and potassium salts.

The carbohydrates, glucose, sucrose, and xylose and the polyhydric alcohols, mannitol, sorbitol, and sorbitan are all effective diuretics when administered intravenously in hypertonic solution. Glucose is the only one of these substances significantly reabsorbed by the renal tubules or significantly metabolized in the body. Thus per gm. administered, it is the least effective, but its lack of toxicity and low cost largely counterbalance this objection. Sucrose, although not metabolized when given intravenously and only slightly reabsorbed by the renal tubules, may cause pathologic alterations in the kidney. Mannitol is inert, only slightly reabsorbed by the renal tubules and effective as a diuretic. Of the organic solutes it is the preferred. However, it must be given intravenously to be effective, and any patient sufficiently ill to justify intravenous therapy can be far more effectively treated in other ways.

Although intravenous sodium sulfate and sodium phosphate have been employed experimentally as osmotic diuretics, they have no place in the therapy of edema. Neither causes a net loss of sodium from the body. In fact both induce a positive sodium balance, for the anion is excreted in part with potassium, hydrogen and ammonia and a part of the sodium is retained in the body.

*Limiting Factors in the Use of Osmotic Diuretics.* For an osmotic diuretic to be effective, osmotically significant quantities of the agent employed must be excreted in the urine. If glomerular filtration rate is low, as it frequently is in edematous states, plasma concentration must be excessively high to deliver adequate quantities of the agent into the proximal urine. Since the capacity of an individual to tolerate a disturbance in osmotic pressure of the body fluids is limited, it may be impossible to obtain significant diuresis with osmotic agents. A second limitation of use is the well recognized fact that mechanisms for conservation of sodium are stimulated in edematous states. Even though greater than normal quantities of ions are delivered into distal portions of the nephron as a result of depression of proximal reabsorption, excessive reabsorption of sodium and chloride as ion pairs and excessive exchange of sodium for hydrogen,

potassium, and ammonia may limit excretion. Osmotic diuretics like all others are effective in the treatment of edema only insofar as they cause loss of body sodium.

## SUMMARY

The normal human kidney can produce very small volumes (0.1 to 0.5 ml. per min.) of highly concentrated urine (1,400 mOsm. per liter) only when the load of osmotically active solutes demanding excretion is low (0.7 mOsm. per min. or less). If large quantities of solutes are excreted as a consequence of their oral or intravenous administration, urine flow increases and urine concentration decreases. If the urinary load of osmotically active solutes is as great as 10 mOsm. per min., urine flow increases to more than 20 ml. per min. and urine osmolality approaches that of the plasma. The excretion of sodium increases more or less in proportion to the increase in urine flow in osmotic diuresis. Increased excretion of sodium and water in osmotic diuresis is the result of decreased proximal tubular reabsorption. The reabsorption of sodium is limited by the inability of proximal tubular cells to pump this ion against a high concentration gradient. The magnitude of this limiting gradient is unknown; it may be of the order of 60 to 80 mEq. per liter. Such reabsorption of sodium and water as does occur concentrates the osmotic diuretic in the proximal tubular fluid. The osmotic effect which it exerts prevents further reabsorption of water. The water retained in the lumen dilutes the sodium and leads to the establishment of a critical gradient against which further transfer of sodium is impossible.

Osmotic diuretics are only moderately effective; hence are not widely employed clinically. Urea and potassium salts are the only osmotic agents which have been used to any extent in the treatment of edema.

## REFERENCES

1. Mudge, G. H., Foulks, J. and Gilman, A.: Effect of urea diuresis on renal excretion of electrolytes. *Am. J. Physiol., 158*:218, 1949.

2. Rapopart, S., Brodsky, W. A., West, C. D., and Mackler, B.: Urinary flow and excretion of solutes during osmotic diuresis in hydropenic man. *Am. J. Physiol., 156*:433, 1949.
3. Rapoport, S, West, C. D., and Brodsky, W. A.: Excretion of solutes and osmotic work during osmotic diuresis of hydropenic man. The ideal and the proximal and distal tubular work; the biological maximum of work. *Am. J. Physiol., 157*:363, 1949.
4. Smith, H. W.: *Principles of Renal Physiology*. New York, Oxford Univ. Press, 1956, p. 237.
5. Wesson, L. G., Jr. and Anslow, W. P., Jr.: Excretion of sodium and water during osmotic diuresis in the dog. *Am. J. Physiol. 153*:465, 1948.
6. Windhager, E. E., Schatzmann, H. J., Whittenbury, G., and Solomon, A. K.: Studies in single proximal tubules of the Necturus kidney. III. The effect of transtubular sodium concentration gradients or of osmotic gradients on water movement. *Am. J. Physiol.*, to be published.
7. Zak, G. A., Brun, C. and Smith, H. W.: The mechanism of formation of osmotically concentrated urine during the antidiuretic state. *J. Clin. Invest., 33*:1064, 1954.

*Chapter XV*

# XANTHINE AND AMINOURACIL DIURETICS

FOR nearly four decades during the latter part of the 19th and first part of the 20th centuries, xanthines were the mainstays of diuretic therapy. Caffeine was first used as a diuretic in a patient with congestive heart failure by Koshlakoff in 1864; theobromine was introduced by the pharmacologist von Schroeder in 1887; and theophylline was studied experimentally by Ach in 1900 and first employed clinically by Minkowsky and Doering in 1903. It was early recognized that theobromine and theophylline are superior to caffeine as diuretics; accordingly, the latter drug has been little used during the present century. In recent years, other more potent drugs have to a large extent replaced xanthines in the intensive diuretic therapy of grossly edematous patients. However, they are still useful in maintenance therapy, for they are adequately absorbed and reasonably well tolerated when given orally. They are most uniquely useful today in the potentiation of mercurial compounds in so-called "diuretic-fast" patients. In this role, aminophylline (theophylline ethylenediamine) excels.

The xanthines as a group exhibit a broad spectrum of pharmacological actions. They differ, however, in the degree to which they individually manifest these several properties. Caffeine is the most powerful of the three as a stimulant of the central nervous system and of skeletal muscle. It is least effective as a diuretic and as a stimulant of the cardiovascular system. Theophylline is the most potent diuretic and the most active stimulant of heart and circulation. It is also highly effective in relaxing the smooth muscle of the biliary tract and of the bronchial tree. It is intermediate in its actions as a central nervous and skeletal muscle stimulant. Theobromine, although less active as a diuretic per unit

weight of drug, can be tolerated in higher dosage than can theophylline, and acts for a longer period of time. Furthermore, it exhibits fewer undesirable side reactions and is, therefore, preferred as a diuretic by some. Recently in a search for improved orally effective compounds, the aminouracils have been observed to have diuretic activity and one, aminoisometridine (Rolicton) may well replace theobromine and theophylline for oral maintenance therapy of edema.

*Chemical Constitution.* Xanthine, as is evident in Figure 29, is a bi-cyclic compound, made up of a 6-membered pyrimidine ring and a 5-membered imidazole ring, the two rings sharing carbons 4 and 5 in common. Caffeine is 1, 3, 7-trimethyl xanthine; theobromine is 3, 7-dimethyl xanthine, and theophylline is 1, 3-dimethyl xanthine. Caffeine occurs naturally in coffee and tea; theobromine in cocoa, and theophylline in tea. The latter two compounds, however, are prepared synthetically, for theophylline, at least, occurs in nature in such small amounts that it is not com-

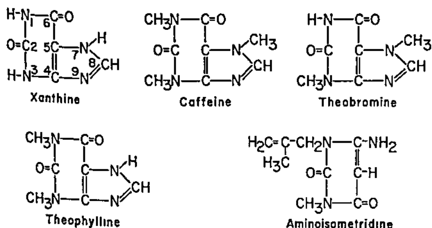



Fig 29. Structure of xanthine and pyrimidinedione diuretics.

mercially feasible to extract. The three compounds are weakly basic alkaloids and are sparingly soluble in water. They form soluble double salts or addition products with citric acid, with sodium glycinate, acetate, and benzoate, and with sodium and calcium salicylates. Theophylline also forms soluble salts with

ethylene diamine, (aminophylline), isopropanolamine, and choline. If the N-7 site of theophylline is substituted with the dihyroxypropyl group or with the diethylaminoethyl group, a water soluble neutral compound results. Aminoisometridine is 1-methallyl-3-methyl-6-amino pyrimidinedione. It is water soluble and administered as the parent compound. Insofar as the xanthines and the aminometridines contain the pyrimidinedione nucleus, they are structurally related.

*Mechanism of Action of Xanthines.* Mudge has recently stated that "the most remarkable thing about the xanthine diuretics is that so few facts have been clearly established concerning the mechanism of their action." This does not imply any early lack of investigative interest, for during the first few decades of this century, many papers, concerned with their mode of action, were published. This literature has been reviewed in extenso by Schmitz and by Vogl. However, the xanthines have been studied with the modern precise tools of renal physiology by relatively few investigators, no doubt because more effective diuretic agents were introduced at the same time that adequate methods of study were developed.

The early German workers, including Veil, Ellinger, Meyer, Ascher, Curtis and others maintained that the xanthines exert their diuretic effects peripherally by altering the binding of water to colloids or by increasing the delivery of chloride and other electrolytes from tissues to blood stream. Such peripheral actions have not been confirmed in recent studies employing more adequate experimental methods. Molitor and Pick ascribed the diuretic action of the xanthines to depression of the hypothalamic center which regulates water metabolism. In view of the fact that xanthines primarily increase the excretion of sodium and chloride and only secondarily affect water output, this view is untenable.

The observation of Schmidt and of Hartwich that caffeine produces diuresis in the isolated perfused frog kidney and that of Gremels and of Verney and Winton that it has a similar action in the Starling heart-lung-kidney preparation of the dog, definitely establishes a direct renal action, although of course it does not rule out other mechanisms as contributory. This view has been con-

firmed by Kupfer *et al.* who have observed that aminophylline causes diuresis in the pump-lung-kidney preparation of the dog perfused at constant pressure.

This direct renal action has been variously ascribed to (1) increased renal blood flow and glomerular filtration rate and (2) to depression of tubular reabsorption of salt and water. Phillips and Bradford first observed that caffeine causes the volume of the kidney to increase and inferred that diuresis results from renal vasodilation. Loewi *et al.* confirmed the observation and concurred in the interpretation. However Gottlieb, Brings, and Cushny as well, have pointed out that swelling of the kidney does not necessarily indicate vasodilation, instead it may reflect constriction of efferent vessels. Furthermore, diuresis occurs at times in the absence of change in kidney volume. Subsequently, more accurate measurements of renal blood flow in animals with the thermo-stromuhr and collection of venous outflow have shown that the xanthines do commonly cause increased renal blood flow. However, the increase in flow is often of relatively short duration and is certainly not a requisite of diuresis.

Richards and his coworkers have shown that caffeine increases the number of patent capillary loops in individual glomeruli and the total number of functioning glomeruli of the frog. Verney and Winton maintain that caffeine increases glomerular capillary filtering pressure and hence filtration rate by dilating afferent glomerular vesels to a greater degree than efferent. Blood flow may either increase, remain constant, or decrease depending on relative efferent tone, yet filtration pressure and filtration rate could, at least theoretically, increase under all circumstances.

Von Schroeder, who first studied the renal action of theobromine, attributed diuresis to stimulation of the secretory activities of renal tubular cells. Barcroft and Gremels supported this view with the observation that the drug increased renal oxygen consumption. However, others have found little evidence of increased renal metabolism, and net tubular secretion of water and salts, other than potassium, is not accepted today. Sobieranski in 1903 was the first to suggest depression of tubular reabsorption of salt and water, but his evidence can scarcely be accepted as significant,

even though subsequent work has amply confirmed his conclusion. Utilizing more or less adequate techniques for measuring glomerular filtration rate and for calculating filtered load of electrolyte, a number of investigators have shown in animals and in man that xanthine diuretics depress tubular reabsorption of sodium, chloride and water. Walker *et al.*, Davenport *et al.*, Blumgart *et al.*, and Crutchfield have emphasized depression of tubular reabsorption and have minimized the significance or denied the occurrence of changes in renal blood flow and filtration rate. However, in somewhat better controlled studies, Newman, Sinclair-Smith *et al.*, James *et al.*, Davis and Shock, and Weston and Escher have provided convincing evidence both of depression of tubular reabsorption and of increase in glomerular filtration. While diuresis may occur in the absence of an increase in filtration rate, it is definitely enhanced, if an increase occurs.

As was pointed out above, theophylline is the most potent cardiovascular stimulant of the xanthine group of drugs. When administered intravenously as aminophylline, cardiac output increases, central venous pressure drops, and oxygenation of blood improves. The drug, therefore, exerts some of the favorable effects of the cardiac glycosides. However, its actions although immediate are evanescent. It appeals to the author that insofar as aminophylline improves the circulatory dynamics of the patient in congestive failure, it will antagonize mechanisms of compensatory fluid retention. Thus it might be expected to increase glomerular filtration rate and reduce excessive secretion of aldosterone indirectly through extrarenal mechanisms responding to improved circulation. Presumably such effects would be mediated through the volume receptor-neurohormonal effector system and would be analogous to those induced by digitalis. Whether they are of significant magnitude is questionable.

Relatively little information is available concerning the mode of action of the aminouracils. Kattus et al have shown that 1-propyl-3-ethyl-6-aminouracil is an effective diuretic in patients with congestive failure, cirrhosis, and nephrosis. In the dog this drug blocks a modest fraction of the tubular reabsorption of sodium and chloride ions without exerting an appreciable effect on either

glomerular filtration rate or renal blood flow. It does not alter acid base balance and causes only a modest loss of potassium. The 1-allyl-3-ethyl analogue is equally effective and less irritating to the gastrointestinal tract. It was marketed briefly as aminometridine (Mictine), to be replaced by the 1-methallyl-3-methyl analogue, *aminoisometridine* (Rolicton), *a compound equally potent, and* accordingly to Clark and Hagans and to Settel, devoid of untoward side reactions.

No information is available concerning the nature of the enzyme systems blocked by either the xanthines or aminouracils, although it is perhaps reasonable to assume that they have a common mode of action. The site of action within the nephron is also unknown. Somewhat more surprising is the lack of information as to factors which modify the response to these agents, eg. acidosis, alkalosis, hyponatremia, hypochloremia etc.

*Dosage and Route of Administration.* Theobromine and theophylline may be administered by rectal suppository, by enema, orally, intramuscularly, and intravenously. Absorption from suppositories is notoriously unpredictable. Futhermore, continued use produces rectal irritation. Absorption of aminophylline by the lower bowel is adequate when the drug is instilled and retained following a cleansing enema.

The oral route is certainly to be preferred for maintenance therapy. However, gastric irritation, anorexia, nausea and vomiting frequently result when diuretically effective doses of the free alkaloids are administered. Theobromine on a weight for weight basis is much less irritating to the gastric mucosa than is theophylline. However, when the two drugs are compared in diuretically equivalent doses (theophylline is roughly 5 times as potent as theobromine), there is little reason to choose one in preference to the other. In general each drug is better tolerated when given in one of the many solubilized forms than when given as the free alkaloid.

Theobromine is usually administered as the sodium salicylate or calcium salicylate complex. Since the effective dosage is high, 3.0 to 5.0 gm. per day in divided doses, and since the sodium content of the sodium salicylate complex is appreciable, the calcium com-

pound is preferred. In general the compounds of theophylline are more widely used today than are those of theobromine because of their slightly greater activity. The free alkaloid, theophylline, in total daily dosage of 0.8 to 1.0 gm. (0.2 gm., 4 to 5 times a day), is probably the most effective oral form in which a xanthine diuretic can be given. However, such usage over any significent period of time is limited by high incidence and severity of gastric irritation. If given with colloidal aluminum hydroxide, gastric tolerance is increased. Less gastric irritation results when theophylline is administered as one of its soluble complexes. These complexes include theophylline sodium glycinate (0.3 gm., 3 to 4 times a day); dihydroxypropyl theophylline (0.2 gm., 3 to 4 times a day); and choline theophyllinate (0.2 gm., 3 to 4 times a day). Theophylline ethylenediamine (0.2 gm., 3 to 4 times a day) is somewhat less well tolerated orally than the above compounds, but irritation can be lessened by coadministration of aluminum hydroxide.

The xanthines are usually administered for periods of 4 consecutive days separated by drug-free intervals of 3 days. The rationale is presumably the following. It is claimed that habituation to xanthines destroys diuretic efficacy and that interrupted courses of treatment avoid this complication. More certain is the fact that gastrointestinal complaints increase in severity more or less in proportion to magnitude of dose and duration of therapy. It is, therefore, advisable to determine the smallest dose capable of maintaining dry weight and to devise some regimen which provides intermittant relief for the digestive tract.

Aminophylline, theophylline sodium glycinate, and certain other preparations are suitable for parenteral administration. In general the intramuscular route is preferred due to slower absorption, more prolonged action, and avoidance of the untoward consequences of too rapid intravenous injection. If the compounds are diluted and given very slowly, intravenous administration has the advantage of avoidance of the pain which follows intramuscular injection. However, too rapid intravenous injection causes giddiness, anxiety, palpitation, tachypnea and hyperpnea followed by nausea, vomiting, and syncope. A few deaths following rapid

intravenous administration have been reported. If the response to oral therapy is inadequate, it would seem wise to turn to more effective agents rather than to resort to intramuscular or intravenous administration of xanthines. An obvious exception to this rule is coadministration of intramuscular mercurial diuretics and either intramuscular or intravenous aminophylline in the treatment of the "diuretic-fast" patient, a procedure discussed subsequently in Chapter XVI in connection with mercurial diuretics.

Aminoisometridine is administered orally in a maintenance dose of 0.2 gm., 2 to 4 times a day. For short periods during the induction of diuresis, as much as 0.5 gm. may be given 3 to 4 times a day. The large dose may produce anorexia and nausea; the smaller maintenance doses produce few or no symptoms. Preliminary studies indicate that the drug is equally or more effective than the theophylline complexes and much better tolerated orally. Further experience will be necessary to assess the true value of aminoisometridine.

*Toxicity.* Signs and symptoms of gastrointestinal irritation from oral administration of xanthines and of systemic intoxication from rapid intravenous administration have been described above. Occasionally signs of excessive central nervous stimulation occur, although they usually constitute no problem with the common therapeutic doses. Theobromine produces less central nervous stimulation than theophylline.

*Indications and Contraindications.* Xanthines and aminoisometridine are on the whole relatively benign drugs, useful in oral maintenance therapy of edematous patients who do not exhibit overly intense salt and water retention. It is stated that the xanthines are most effective in the congestive failure of arteriosclerotic and hypertensive heart disease and less effective in rheumatic heart disease, cirrhosis with ascites, nephrosis and the nephrotic stage of chronic nephritis. They are frequently effective in the treatment of pre-eclampsia and premenstrual edema and tension. Aminoisometridine has not been used for a sufficient period to define its therapeutic limits, although it is probable that they are much the same as those of the xanthines. For several decades the xanthines were the only agents available for use in

severely ill patients intolerant to mercurial diuretics, and were given parenterally in such instances. Now there is a wider choice of drugs suitable for use in mercury intolerant patients and indications for parenteral therapy are less frequent.

Intolerance to xanthine diuretics is rare and manifestations of hypersensitivity are mild. The intravenous use of aminophylline is to be avoided in patients with recent extensive myocardial infarcts because the drug stimulates the myocardium intensely and may induce arrhythmias. The xanthines reduce clotting time and prothrombin time. It is possible that they might promote phlebothrombosis in severely ill or elderly patients, although there is no evidence that this is so. Xanthines depress the hepatic conversion of ammonia to urea in vitro. They probably should not be administered to patients with marked hepatic insufficiency because of the possibility of precipitating liver coma.

## SUMMARY

Theophylline in one of its numerous solubilized forms is a useful drug for the oral maintenance therapy of edematous patients. Because it is somewhat more effective than theobromine, it is probably the drug of choice. The xanthines in general depress a modest fraction of the renal tubular reabsorption of sodium, chloride and water, increase the excretion of potassium slightly, have no effect on urine pH and ammonia excretion, and cause no significant disturbance in acid base balance. Theophylline frequently increases renal blood flow and glomerular filtration rate, especially in those patients without organic renal disease in whom these discrete functions are depressed. When filtration rate is increased, the diuretic response is enhanced. The aminouracils have similar renal actions except that they have little or no effect on glomerular filtration rate or renal blood flow.

Oral administration of theophylline in diuretically effective doses is frequently limited by gastrointestinal irritation. Use of the more benign solubilized forms or coadministration of colloidal aluminum hydroxide reduces irritation and permits more effective therapy. Aminoisometridine is largely devoid of irritating properties. Xanthines and aminouracils are most effective in those

patients who mildly retain salt and water. For more severely ill patients, requiring intensive parenteral therapy, other agents are more effective. In such patients, mercurial diuretics, potentiated by acidifying agents and by intramuscular or intravenous aminophylline, are especially effective.

## REFERENCES

1. Clark, M. L. and Hagans, J. A.: The diuretic activity of a new pyrimidinedione administered orally to non-edematous adults. *J. Lab. & Clin. Med., 49*:395, 1957.
2. Davis, J. O. and Shock, N.: The effect of theophylline ethylenediamine on renal function in control subjects and in patients in congestive faliure. *J. Clin. Invest, 28*:1459, 1949.
3. Hyman, J. M., Jr.: Clinical use of diuretics. *J.A.M.A., 107*:1937, 1936.
4. Schmitz, H. L.: Studies on the action of diuretics. I. The effect of euphyllin and salyrgan upon glomerular filtration and tubular reabsorption. *J. Clin. Invest., 11*:1075, 1932.
5. Vogl, A.: *Diuretic Therapy*. Baltimore, Williams & Wilkins, 1953.
6. Settel, E., Rolicton (aminoisometridine) a new non-mercurial diuretic. *Postgraduate Med, 21*:186, 1957.

*Chapter XVI*

# MERCURIAL DIURETICS

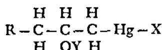
ORGANOMERCURIAL diuretics rank among the most valuable of the chemotherapeutic agents in use today. They have contributed immeasurably to the comfort, useful existence, and life span of countless patients over a period of nearly forty years. They are the time-proven standard of reference against which other diuretic agents are compared as to efficacy, reliability and toxicity. However, as is true of all potent drugs, certain risks attend the use of mercurial diuretics, risks which may be minimized by careful attention to such details of therapy as dose, route of administration, frequency of exhibition, and contraindications.

Mercury has an ancient, if not entirely venerable history as a diuretic. Paracelsus, early in the 16th Century, described the use of calomel as a purgative and diuretic. Various mixtures of mercury, calomel, digitalis, and squill were employed in the treatment of congestive heart failure as recently as the first decades of the present Century. However, mercurialism, with its attendant enteritis, stomatitis, renal damage, and occasional fatal outcome, discouraged any very intensive diuretic therapy with inorganic compounds of mercury.

Organomercurial compounds were first introduced as antisyphilitic agents, not as diuretics. In 1920, Saxl and Heilig described polyuria following each injection of Novasurol during the course of treatment of a patient for congenital syphilis. Subsequently, the diuretic efficacy of this drug was demonstrated in patients with congestive heart failure of rheumatic origin. Over the past 40 years a succession of organomercurial compounds have been produced, of increasing diuretic efficacy and diminishing toxicity. When carefully and conservatively used, the organomercurial compounds available today do not induce mercurialism.

However, fear of this eventuality has no doubt deprived many patients of the benefits of what as a class are the most effective of all diuretics.

***Chemical Nature of Mercurial Diuretics.*** The mercurial diuretics in common use today as well as those undergoing clinical trial are substituted mercuripropyl compounds having the following basic structure.




The most significant feature of the diuretic structure is the terminal —C—$Hg^+$ linkage which in neutral or alkaline solution is quite stable. The carbon mercury bond does not dissociate reversibly to give mercuric ion; if ruptured in acid solution, this bond does not spontaneously reform. The —Hg—X linkage, in contrast, is an ionic one; on dissociation, one mercury valence bond is freed to combine with tissue components. In 5 of the 7 drugs currently listed in New and Nonofficial Remedies, X is theophylline, in one, it is chloride; and in one, it is thioacetic acid. Theophylline, as the X substituent, increases solubility of the drug, decreases local irritation at the site of intramuscular deposition, and increases rate of absorption from that site. Whether it increases rate of urinary excretion and diuresis except by virtue of the fact that it increases absorption, is debatable, for the amount of theophylline contained in the usual therapeutic dose is relatively small. A striking decrease in cardiotoxicity and a further reduction in local irritation is observed when the X moiety is thioacetic acid as in mercaptomerin In chlormerodrin, X is chloride. This compound, while reasonably well absorbed from the gut, is irritating when given parenterally, accordingly, it is recommended for oral use only.

The OY substituent on the propyl chain is most commonly $OCH_3$ (methoxy). Its nature is of relatively minor importance in determining diuretic activity and either local or systemic toxicity. Its character is related to the solvent in which the mercuration reaction is carried out, and since the solvent most commonly employed is methyl alcohol, the methoxy substituent is the usual one.

The major structural differences among the several diuretics listed in Table X are evident in the R substituent. This grouping is allicyclic in mercurophylline and mercaptomerin; it is aromatic in mersalyl and merethoxylline; it is heterocyclic in mercumatilin; and it is acyclic in meralluride and chlormerodrin. In 6 of the 7

TABLE X

STRUCTURE OF THE COMMONLY USED ORGANO-MERCURIAL DIURETICS

| ORGANOMERCURIAL COMPOUND | COMPLEXING AGENT | NAME |
|---|---|---|
| NaO–C(=O)–[ring: $CH_3$, $CH_3$, $CH_3$]–C(=O)–NH–$CH_2$–CH(O–$CH_3$)–$CH_2$–HgOH | Theophylline | MERCUROPHYLLINE SODIUM (Mercuzanthin) |
| | Sodium Thioocetate | MERCAPTOMERIN SODIUM (Thiomerin Sodium) |
| [benzene ring]–C(=O)–NH–$CH_2$–CH($OCH_3$)–$CH_2$–HgOH; O–$CH_2$–C(=O)–O–Na | Theophylline | MERSALYL SODIUM AND THEOPHYLLINE (Salyrgan–Theophylline) |
| [benzene ring]–O–$CH_2$–C(=O)–O–Hg; C(=O)–NH–$CH_2$–CH(O–$CH_2$–$CH_2$–$OCH_3$)–$CH_2$ | Theophylline and Procaine | MERETHOXYLLINE PROCAINE (Dicurin Procaine) |
| $CH_2$–CH($OCH_3$)–$CH_2$–HgOH on [coumarin ring, =O]–C(=O)–ONa | Theophylline | MERCUMATILIN SODIUM (Cumertilin Sodium) |
| (ONa)C(=O)–$CH_2$–$CH_2$–C(=O)–NH–C(=O)–NH–$CH_2$–CH($OCH_3$)–$CH_2$–HgOH | Theophylline | MERALLURIDE SODIUM (Mercuhydrin Sodium) |
| $NH_2$–C(=O)–NH–$CH_2$–CH($OCH_3$)–$CH_2$–Hg–Cl | — | CHLORMERODRIN (Neohydrin) |

compounds listed, the linkage between R and the propyl side chain is amide; in one it is carbon to carbon. At least one compound undergoing clinical trial has an ether linkage. R is by far the most important determinant of diuretic activity and toxicity. However, since the drugs accepted for clinical use have been selected from large numbers of compounds screened, it is not surprising that they are reasonably comparable so far as potency and toxicity are concerned. Furthermore, no pattern of R-structure has yet emerged which would enable one to design a compound of predictable properties. Practically any substituted allyl

compound ($R-CH_2 \cdot CH:CH_2$) on mercuration in methyl alcohol will exhibit diuretic properties. Whether clinically useful must be determined emperically. It should, however, be pointed out that many, perhaps most, organic compounds of mercury are not diuretics. Subsequently, we shall consider two divergent views as to why certain compounds of mercury are diuretics, why others are not.

*Renal Action of Diuretics.* For some ten years after their introduction, *organic mercurial diuretics were thought to exert their* action by mobilizing salt and water in the tissues. Govaerts in 1928 observed that if one kidney of a dog is removed at the peak of a mercurial diuresis, and if its blood vessels are anastomosed with those of a non-diuretic animal, the transplanted kidney continues to exhibit polyuria. Bartram in 1932 noted that a minute dose of a mercurial diuretic, introduced into one renal artery, causes a prolonged increase in the excretion of urine by that kidney alone. A large dose, however, causes renal shutdown on the side injected and diuresis in the opposite kidney. These simple experiments demonstrated three fundamental facts concerning the action of mercurial diuretics: (1) they act directly on the kidneys; (2) they are fixed in renal tissue in the course of a single transit through the renal vascular bed and exert a prolonged diuretic effect; (3) in large dosage they are toxic and may cause renal shutdown. Such experiments do not exclude the possibility of peripheral action, but the observation of hemodilution prior to onset of diuresis, upon which this view was based, has not been confirmed in more recent studies.

*Glomerular vs. Tubular Site of Action.* A summary of a rather sophisticated modification of Bartram's experiment is presented in Figure 30. The two ureters of an anesthetized dog were separately catheterized and the animal was infused with creatinine to permit measurement of glomerular filtration rate. Following two control periods, shown at the left of the figure, a small dose of chlormerodrin labelled with radioactive Hg-203 was injected into the left renal artery. After a delay of 10 minutes, a brisk diuresis of sodium and water occurred which was restricted to the left kidney for some 60 to 75 minutes. Since diuresis developed without change

in filtration rate, it must have resulted from inhibition of tubular reabsorption of fluid and electrolyte. Uptake of the diuretic by the left kidney was incomplete; some obviously escaped into the general circulation, as evidenced by the appreciable concentrations of mercury in femoral arterial plasma. This accounts for the

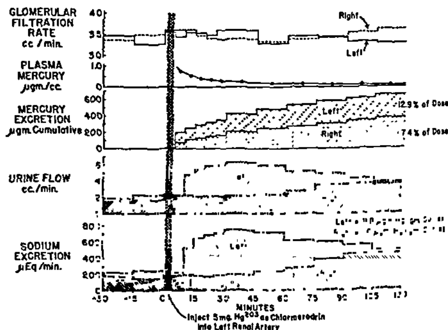



Fig 30. The diuretic response to the administration of 5 mg. of $Hg^{203}$ as chlormerodrin into the left renal artery of a dog. (From R.F. Pitts: *Am. J. Med.*, 24:745, 1958.)

accumulation of some of the diuretic in the right renal cortex, for its excretion in the urine formed by the right kidney, and for delayed diuresis which gradually developed on the right side. Evidence from a variety of sources indicates that mercurial diuresis results primarily from depression of tubular reabsorption of fluid and electrolyte, not from increase in filtered load.

*Extent of Tubular Depression of Ion Reabsorption.* In clinical practice the maximum dose of a mercurial diuretic, administered in a single injection, does not ordinarily exceed 2 ml. and contains not more than 85 mg. of mercury. For the average adult patient this represents slightly more than 1.0 mg. of mercury per Kg. of

body weight. Farah has observed that diuresis and natriuresis in dogs increase with dosage over the range of 0.5 to 5.0 mg. of mercury per Kg. Maximum excretion of sodium was obtained with doses between 3.0 and 5.0 mg. per Kg. and further increases to 25 mg. per Kg. did not enhance the response. While studies over such an extended range are out of the question in man, limited observations indicate that peak effects are obtained with 2.0 to 4.0 mg. of mercury per Kg.; thus man and dog exhibit roughly comparable sensitivities to the diuretic effects of these drugs. Since toxicity is related to dosage, even quantities of 2.0 to 4.0 mg. per Kg. cannot be justified in therapy.

The significant finding of these studies is that only a limited fraction of tubular reabsorption of sodium and chloride can be blocked by even the largest tolerated doses of drug. This fact is clearly illustrated in the data summarized in Table XI. In this experiment a dog was infused with isotonic saline at a rate of 10 ml. per min. for a period of 2½ hr. prior to, and throughout the course of the observations. Such extensive hydration, by expanding extracellular fluid volume, makes certain that diuresis will not be restricted by limited salt and water reserves. It also accounts for the high rate of urine flow and high rate of sodium excretion in the two initial control periods. During these periods, 93 per cent of the filtered sodium was reabsorbed, 7 per cent was excreted. A dose of 100 mg. of mercury as Mercuhydrin was then given intravenously. Urine flow increased from 9 to 17 ml. per min. and sodium reabsorption dropped from 93 to 80 per cent of that filtered, a decrease of 13 per cent. The dose of mercury was roughly 5.0 mg. per Kg. body weight, some 5 times the therapeutic dose in man, and sufficient to produce maximum depression of sodium reabsorption.

In other similar experiments maximum depression has varied between 12 and 20 per cent .Obviously, a large fraction, some 80 per cent or more of tubular reabsorption of sodium, is resistant to the action of mercurial diuretics. Blockade of only 20 per cent of sodium reabsorption might be related to depression of transport in a limited segment of the renal tubule, perhaps in the terminal part of the proximal segment. It might also be related to inhibition of

in filtration rate, it must have resulted from inhibition of tubular reabsorption of fluid and electrolyte. Uptake of the diuretic by the left kidney was incomplete; some obviously escaped into the general circulation, as evidenced by the appreciable concentrations of mercury in femoral arterial plasma. This accounts for the

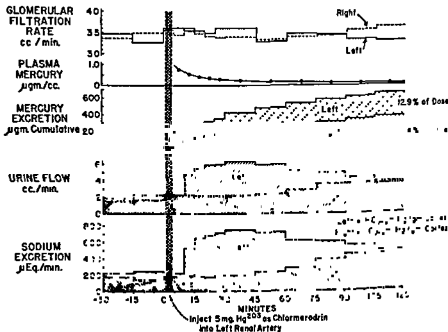



Fig. 30. The diuretic response to the administration of 5 mg. of Hg[203] as chlormerodrin into the left renal artery of a dog. (From R.F. Pitts. *Am. J. Med.*, 24 745, 1958.)

accumulation of some of the diuretic in the right renal cortex, for its excretion in the urine formed by the right kidney, and for delayed diuresis which gradually developed on the right side. Evidence from a variety of sources indicates that mercurial diuresis results primarily from depression of tubular reabsorption of fluid and electrolyte, not from increase in filtered load.

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an enzyme system which normally supplies a limited fraction of the energy for sodium transport throughout the proximal tubule. The author favors this latter view. Diuretic responses comparable to those shown in Table XI are not seen clinically, nor would they be desirable, for they would result in the precipitous discharge of excessive volumes of edema fluid, reduction in circulating blood volume, and circulatory collapse. At the end of this experiment 100 mg. of British Anti-Lewisite (BAL) was given intramuscularly. This substance complexes the diuretic and inhibits diuresis. Its mechanism of action will be considered later.

*Effects on Water Reabsorption.* It seems fairly certain that increased urine flow following mercurial diuretics is the osmotic consequence of inhibition of proximal tubular reabsorption of sodium and chloride ions (vide infra). It is not the consequence of direct interference with tubular mechanisms responsible for the formation of hypertonic urine, namely with ion pumps in the loops of Henle and with facultative control of the permeability of the distal tubules and collecting ducts to water. In other words mercurials do not block the mechanism for concentrating the urine, they merely render it less effective by increasing the osmotic load of salt. Thus Brodsky has observed identical relationships between urine flow and urinary osmotic load in hydropenic dogs infused with hypertonic mannitol and those given mercurial diuretics. Capps has noted in maximally hydrated normal subjects, exhibiting both water diuresis and mercurial diuresis, that infusion of antidiuretic hormone produces a decrease in urine flow and an increase in urine osmolality similar to that produced in control subjects exhibiting water diuresis alone. Thus the renal mechanisms which respond to endogenous and exogenous antidiuretic hormone seem basically unaffected by mercurial diuretics.

*Blockade of Reabsorption of Sodium vs. Chloride.* A fair amount of attention has been devoted to the questions: do mercurial diuretics primarily block chloride or sodium reabsorption? If as many claim, chloride reabsorption is specifically blocked, is increased sodium excretion merely a consequence of the necessity for eliminating in the urine equal numbers of cations and anions? Most would answer these questions in the affirmative for the

TABLE XI
THE DIURETIC EFFECT OF A LARGE DOSE OF MERCUHYDRIN IN THE DOG AND THE ANTIDIURETIC EFFECT OF BAL

| *Urine Flow* | *Glom. Filt. Rate* | *Plasma Sodium* | *Urine Sodium* | *Sodium* | | | |
|---|---|---|---|---|---|---|---|
| | | | | *Filtered* | *Excreted* | *Reabsorbed* | |
| *(ml./min.)* | *(ml./min.)* | *(mEq./L.)* | *(mEq./L.)* | *(mEq./min.)* | *(mEq./min.)* | *(mEq./min.)* | *(% filtered)* |
| *10 ml. Saline per min. for preceding 2 hrs. and 30 min. intravenously* | | | | | | | |
| 8.93 | 80.0 | 151 | 90.5 | 11.49 | 0.81 | 10.68 | 93.0 |
| 9.07 | 81.7 | 152 | 89.7 | 11.81 | 0.81 | 11.00 | 93.1 |
| *100 mg. Hg as Mercuhydrin intravenously* | | | | | | | |
| 11.58 | 86.5 | 152 | 114 | 13.16 | 1.33 | 11.83 | 89.8 |
| 11.52 | 85.7 | 153 | 129 | 12.44 | 1.47 | 10.97 | 88.3 |
| 15.60 | 83.0 | 154 | 126 | 12.10 | 1.96 | 10.14 | 84.8 |
| 16.86 | 83.8 | 152 | 135 | 12.11 | 2.28 | 9.83 | 81.1 |
| 17.06 | 83.4 | 153 | 137 | 12.10 | 2.34 | 9.76 | 80.6 |
| 16.53 | 80.4 | 153 | 142 | 11.60 | 2.33 | 9.27 | 79.9 |
| *100 mg. BAL intramuscularly* | | | | | | | |
| 10.33 | 71.3 | 155 | 139 | 9.82 | 1.44 | 8.38 | 85.3 |
| 5.40 | 85.2 | 156 | 125 | 12.60 | 0.67 | 11.93 | 94.7 |
| 6.33 | 82.5 | 157 | 128 | 12.39 | 0.81 | 11.58 | 94.3 |

(From J. J. Duggan and R. F. Pitts: *J. Clin. Invest.*, 29:365, 1950.)

both dog and man, are rapidly removed from the blood stream and concentrated in the kidneys; more specifically in the renal cortex. An experiment of Borghgraef is summarized in Table XII. Two dogs were given 1.0 mg. of mercury per Kg. intravenously in the form of Neohydrin. To facilitate analysis, the diuretic was synthesized with radiomercury $Hg^{203}$. Two hours later, at the peak of diuresis blood samples were drawn, the animals were sacrificed, and portions of representative tissues were removed. During the two hour period of diuresis, each of the two dogs excreted in the urine 40 per cent of the dose administered. The plasma concentrations of mercury were low, roughly 1.0 microgram ($\mu$gm.) per ml.

TABLE XII

Comparison of the Distribution of Chlormerodrin in the Kidney and in Representative Tissues of the Dog

| | *Dog A* | | *Dog B* | |
|---|---|---|---|---|
| *Tissue* | *µgm Hg/gm. or/ml.* | *Tissue/ Plasma* | *µgm. Hg/gm. or/ml.* | *Tissue/ Plasma* |
| Plasma | 0 91 | —— | 1 02 | —— |
| Kidney | | | | |
| Cortex | 163 | 179 | 131 | 128 |
| Papilla | 2.17 | 2.38 | 3.38 | 3.31 |
| Liver | 2.82 | 3.10 | 2.30 | 2.26 |
| Spleen | 1 93 | 2.12 | 0 86 | 0 84 |
| Intestine | 0 71 | 0.78 | —— | —— |
| Adrenal | 0 51 | 0.56 | 1.66 | 1.63 |
| Heart | 0.27 | 0 30 | 0.29 | 0.28 |
| Muscle | 0 16 | 0 18 | 0.11 | 0.11 |
| Excretion in 2 hours | 40 3% of dose | | 40.8% of dose | |

(From R.R.M. Borghgraef and R. F. Pitts· *J. Clin. Invest.*, 35.31, 1956)

following reasons. Edematous patients undergoing mercurial diuresis commonly excrete more chloride than sodium in the urine, the sodium deficit being made up by potassium and ammonia. In the course of repeated diureses, *loss of body chloride* in excess of sodium results in hypochloremic alkalosis. If the plasma chloride drops below 90 to 95 mEq. per liter and if the plasma bicarbonate rises above 30 to 35 mEq. per liter, mercurial diuretics become ineffective. If normal chloride concentration is restored by the *administration of ammonium chloride, responsiveness to mercurial* diuretics returns. If hyperchloremic acidosis is induced, the diuretic activity of mercurials is markedly enhanced.

Strangely these observations do not prove primacy of blockade of chloride reabsorption. As Weston, Berliner and others have *pointed out, they are compatible with primary blockade of sodium reabsorption in the proximal tubule. If less sodium is reabsorbed* proximally, more sodium and hence more chloride will be delivered into the distal tubules and collecting ducts. If the mechanisms which exchange hydrogen, potassium and ammonia for *sodium are stimulated, as they appear to be in edematous patients,* less sodium than chloride will be excreted and the sodium deficit will be made up largely by hydrogen (more acid urine), potassium, and ammonia. If, as implied in Chapter IV, sodium transport is active and chloride transport passive, mercurial diuretics would *of necessity block sodium rather than chloride reabsorption.* This view is consistent with the finding of Giebisch that mercurial diuretics partially depolarize proximal tubular cells, i.e., lower transcellular potentials (see Chapter IV), presumably by slowing the rate at which the sodium pump ejects sodium from the cell *into the peritublar fluid. However, there is no incontrovertible* evidence in favor of this thesis. There may exist an independent active transport mechanism for chloride, and mercurials may block *it. However, the author is inclined now, although not previously,* to view sodium reabsorption as active, and in the proximal tubule, to be subject to partial blockade by mercurial diuretics.

*Distribution of Mercurial Diuretics in the Body.* It has been shown by Threefoot, Weston, Borghgraef and their respective associates, that mercurial diuretics, administered intravenously in

both dog and man, are rapidly removed from the blood stream and concentrated in the kidneys; more specifically in the renal cortex. An experiment of Borghgraef is summarized in Table XII. Two dogs were given 1.0 mg. of mercury per Kg. intravenously in the form of Neohydrin. To facilitate analysis, the diuretic was synthesized with radiomercury $Hg^{203}$. Two hours later, at the peak of diuresis blood samples were drawn, the animals were sacrificed, and portions of representative tissues were removed. During the two hour period of diuresis, each of the two dogs excreted in the urine 40 per cent of the dose administered. The plasma concentrations of mercury were low, roughly 1.0 microgram (μgm.) per ml.

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| | *Dog A* | | *Dog B* | |
|---|---|---|---|---|
| *Tissue* | *μgm. Hg/gm. or/ml.* | *Tissue/Plasma* | *μgm. Hg/gm. or/ml.* | *Tissue/Plasma* |
| Plasma | 0.91 | —— | 1.02 | —— |
| Kidney | | | | |
| Cortex | 163 | 179 | 131 | 128 |
| Papilla | 2.17 | 2.38 | 3.38 | 3.31 |
| Liver | 2.82 | 3.10 | 2.30 | 2.26 |
| Spleen | 1.93 | 2.12 | 0.86 | 0.84 |
| Intestine | 0.71 | 0.78 | —— | —— |
| Adrenal | 0.51 | 0.56 | 1.66 | 1.63 |
| Heart | 0.27 | 0.30 | 0.29 | 0.28 |
| Muscle | 0.16 | 0.18 | 0.11 | 0.11 |
| Excretion in 2 hours | 40.3% of dose | | 40.8% of dose | |

(From R.R.M. Borghgraef and R. F. Pitts. *J. Clin. Invest.*, 35 31, 1956.)

following reasons. Edematous patients undergoing mercurial diuresis commonly excrete more chloride than sodium in the urine, the sodium deficit being made up by potassium and ammonia. In the course of repeated diureses, loss of body chloride in excess of sodium results in hypochloremic alkalosis. If the plasma chloride drops below 90 to 95 mEq. per liter and if the plasma bicarbonate rises above 30 to 35 mEq. per liter, mercurial diuretics become ineffective. If normal chloride concentration is restored by the *administration of ammonium chloride, responsiveness to mercurial* diuretics returns. If hyperchloremic acidosis is induced, the diuretic activity of mercurials is markedly enhanced.

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*Distribution of Mercurial Diuretics in the Body.* It has been shown by Threefoot, Weston, Borghgraef and their respective associates, that mercurial diuretics, administered intravenously in

tained. Kessler has shown that at least the last two of these postulates are not strictly true in the experimental animal. Thus diuresis wanes during the maintenance of the plateau of cortical concentration, no doubt limited in part by exhaustion of readily available extracellular fluid reserves. Perhaps the volume receptor system

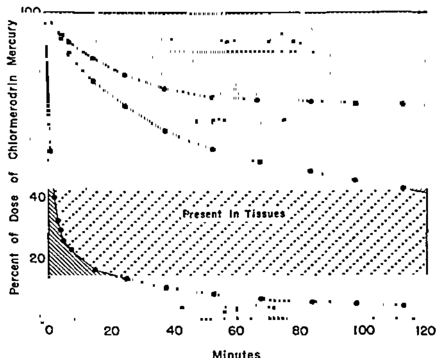



Fig. 31. The distribution in the body and urinary excretion of chlormerodrin by the dog as a function of time following the intravenous administration of 1.0 mg of $Hg^{203}$ per Kg. as diuretic drug. (From R.R.M. Borghgraef, R.H. Kessler and R.F. Pitts *J. Clin Invest.*, 35:1055, 1956)

operates to protect dwindling fluid reserves by reducing filtration rate and stimulating mercury resistant reabsorptive systems. Furthermore, Kessler has shown that certain non-diuretic organic compounds of mercury are concentrated in the renal cortex to a degree comparable to that to which the diuretics are concentrated. Apparently such simple explanations of onset, intensity and duration of mercurial diuresis as those proposed at the beginning of this paragraph are inadequate.

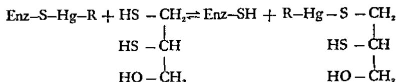
in the two experiments. In contrast, the concentrations of mercury in the renal cortex were high, 163 and 131 $\mu$gm. per gm. The specificity of binding by the cortex is illustrated by the fact that the concentrations of mercury in the renal papillae were only 2.2 and 3.4 $\mu$gm. per gm., considerably less than in the urine formed just prior to sacrifice. Tissue/plasma ratios indicate the degree of concentration in the several tissues relative to that in an equivalent amount of plasma. It is evident that only the renal cortex concentrates the drug appreciably.

Greif, using methods of homogenization and differential centrifugation, has shown that a part of the diuretic bound by the renal cortex is combined with mitochondria to form a stable nondializable complex. However, most of the renal mercury is bound to soluble cytoplasmic proteins of cortical cells.

It is possible, by catheterizing the renal vein, by simultaneously collecting arterial blood, renal venous blood and urine, and by serially measuring renal blood flow, to describe the distribution in the body of a mercurial diuretic moment by moment after it is introduced into a peripheral vein. Such a description for a dog given 1 mg. of mercury per Kg. as chlormerodrin is summarized in Figure 31, taken from the work of Borghgraef. At zero time, i.e., immediately after introduction into a vein, the entire dose of the diuretic is contained in the plasma compartment, for little penetrates red cells. It immediately distributes into tissue; accordingly, plasma concentration falls rapidly during the first 20 mins. The kidney removes the drug from the peritubular blood, concentrates it within the renal cortex and secretes it into the urine. After 40 to 60 mins. the concentration in the renal cortex reaches a steady state: the diuretic is delivered from general tissue reservoirs to the kidney as rapidly as the kidney secretes it into the urine. Studies of Weston et al and Threefoot et al indicate that the distribution of Mercuhydrin in man follows a similar pattern and time course.

It is tempting to relate the onset of diuresis to the development of some critical concentration of diuretic in cortical tissue; the intensity of diuresis to the plateau concentration attained, and the duration of diuresis to the time some critical concentration is main-

integrity of sulfhydryl groups. This reaction is described in the following equation,



$$\begin{array}{lcl} \text{Enz–S–Hg–R} + \text{HS}-\text{CH}_2 & \rightleftharpoons & \text{Enz–SH} + \text{R–Hg}-\text{S}-\text{CH}_2 \\ \quad\quad\quad\quad \text{HS}-\text{CH} & & \quad\quad\quad\quad \text{HS}-\text{CH} \\ \quad\quad\quad\quad \text{HO}-\text{CH}_2 & & \quad\quad\quad\quad \text{HO}-\text{CH}_2 \end{array}$$

Inhibition of mercurial diuresis by BAL is illustrated in the last three clearance periods of the experiment presented earlier in Table XI.

*Excretion of Mercurial Diuretics.* Parenterally administered mercurial diuretics are eliminated largely by the kidneys, to a minor degree by the bowel, and to negligible extent in saliva, sweat and milk. Fecal excretion accounts for one-third or less of total excretion; the bulk of this moiety enters the gut in the bile. When mercurials are administered per os, a higher proportion is eliminated in the feces, for intestinal absorption of most of these drugs is relatively poor. Neohydrin and Cumertilin stand apart, in that they are better absorbed on oral administration.

Rate of urinary excretion of drug depends on route of administration, and is highest after intravenous injection, somewhat slower after intramuscular and subcutaneous injection and slowest after oral administration Combination of the drug with theophylline or thioacetic acid as the X substituent increases solubility, increases rate of absorption from the local site of deposition, and therefore increases rate of excretion. Estimates of the rate of excretion of the several accepted preparations by edematous patients range from 60 to 100 per cent of a single therapeutic dose in 24 hours. As much as 50 per cent may be excreted in 3 to 6 hours. Rarely is recovery of the administered dose complete, no doubt in fair part due to capricious chemical methods. It is certain that the vast majority of patients, given repeated injections, do not accumulate the drugs. However, it is equally certain that patients who do not give an adequate diuretic response, who are oliguric or anuric, or who have marked renal insufficiency and nitrogen retention do not excrete the drugs readily and may show cumulative toxicity if

*Enzyme Inhibition in Diuresis.* One of the most characteristic reactions of inorganic salts of mercury and of those organic compounds with one free mercury valence is the formation of mercaptides with thiols. It is a reasonable assumption to assign the diuretic properties of mercurial compounds to their ability to inhibit renal sulfhydryl enzymes by forming inactive mercaptide complexes. Wachstein and Meisel, Rennels and Ruskin, and others have noted that the activity of succinic dehydrogenase, demonstrable histochemically in the kidney of the rat by the neotetrazoleum reaction, is inhibited by the prior administration of mercurial diuretics in extremely high dosage (10 to 30 mg. of mercury per Kg. body weight). The inhibition is most pronounced in the third or straight descending segment of the proximal tubule. Unfortunately, doses equivalent to therapeutic doses in man have no demonstrable effect on succinic dehydrogenase activity. Therefore, inhibition of the enzyme may have more pathological than functional significance. Cafruny, *et al.*, using quantitative methods for histochemical identification of protein bound sulfhydryl groups in sections of kidney, have observed reductions in concentration in proximal tubules, loops of Henle, and collecting ducts following the administration of mercurial diuretics in amounts within the therapeutic range. It is possible that among these protein bound sulfhydryl compounds are enzymes which supply energy to the machinery which transports sodium ions.

The following equation, Enz-SH + $^{+}$Hg-R ⇄ Enz-S-Hg-R, implies that a mercurial diuretic ($^{+}$Hg-R) reacts reversibly with sulfhydryl enzymes, to yield inactive complexes. These enzymes have a greater affinity for diuretics than do monothiols such as cysteine and glutathione, but a lesser affinity than do dithiols such as BAL. This statement derives from the observation of Earle, Farah and others that mercurial compounds complexed with monothiols retain their diuretic properties; those complexed with dithiols do not. Dithiopropanol, BAL, administered at the peak of a mercurial diuresis, promptly reduces salt excretion and urine flow to the control range by complexing the diuretic and restoring the

dryl compounds. The same statement applies to the free valence of the mercury linked to the terminal carbon of the parent molecule; it too must be largely bound by thiols. The thesis that rupture of the carbon-mercury bond is a requisite of diuretic activity has recently been revived by Mudge and his colleagues. Mudge has observed a relationship between *in vivo* diuretic activity and *in vitro* acid lability in a series of organomercurial compounds. He explains the well recognized potentiation of mercurial diuresis by ammonium chloride in terms of the intracellular as well as extracellular acidosis which it induces. The greater the renal intracellular acidosis, the more the parent compound is broken down to diuretic mercuric ion. Inhibition of diuresis in metabolic alkalosis is presumed to result from greater stability of the parent compound. In view of Müller and Weiner's results, namely that most of the drug excreted in the urine is in the form of parent compound complexed with cysteine or acetyl cysteine, it is necessary to make the following assumption. Only a minute fraction of the drug taken up by the kidney, concentrated in the cortex, and secreted in the urine is diuretically active. The bulk of the diuretic serves no pharmacologically useful purpose; only the minute fraction which is broken down is active. In support of their thesis, Mudge et al have shown that the diuretic efficacy of Mercuhydrin (an acid-labile compound) is markedly affected by alterations in acid base balance; the efficacy of mercuric cysteine (an ionizable compound) is not. Furthermore, per mg. of mercury administered, the diuretic activity of mercuric cysteine[1a] is considerably greater than that of Mercuhydrin, for all of its mercury is potentially available as mercuric ion.

While this thesis has its attractive aspects and may well be true, certain facts argue against it at the moment. The infusion of acetazoleamide and of potassium chloride, procedures which not only alkalinize the urine but presumably elevate the pH of the contents of renal tubular cells as well, do not alter the diuretic response

[1a]This should not be construed as indicating that mercuric cysteine is a clinically useful diuretic; it is not, for it is highly toxic.

doses are repeated at short intervals in an attempt to force a response.

Mercurial diuretics are eliminated in the urine almost entirely by a process of active tubular secretion. Several lines of evidence point to this conclusion. Diuretics circulating in the blood stream are highly bound to the SH groups of plasma albumin. To the extent that they are bound, they are non-filterable through glomerular capillaries. The albumin-diuretic complex must however dissociate to some extent, for during passage of blood through peritubular capillaries, the drug is transferred to tubular cells. Tubular cells must therefore have a greater affinity for mercurial diuretics than do plasma proteins. Muller and Weiner have shown that mercurial diuretics are eliminated in the urine as complexes of the parent compound with cysteine or acetyl cysteine. Obviously, energy must be expended in breaking the strong linkage between diuretic and tubular cell in order to complex it with a monothiol to which it is less firmly bound, and transfer it into the urine. Calculations of Borghgraef, from the arterio-venous extraction studies mentioned previously, indicate that not less than 90 per cent and probably more nearly 100 per cent of urinary diuretic is eliminated by a process of active tubular secretion. It seems certain that a variety of organic compounds of mercury, having widely different structures, and inorganic mercuric ions as well are secreted into the urine as monothiol complexes by a common tubular mechanism. It is, therefore, likely that the carrier system combines with the free —$Hg^{+}$ valence common to all. Perhaps the transport mechanism is a basic one devised to rid the body of the traces of heavy metals which are contained in ingested food and water. The older concept that both excretion and diuretic activity can be explained in terms of glomerular filtration and partial tubular reabsorption of the mercurial compounds is patently incorrect.

*The Mechanism of Enzyme Inhibition.* Sollman some years ago postulated that organic compounds of mercury are diuretics by virtue of the fact that they decompose in the body to liberate mercuric ions. Of course mercuric ions exists in the body in vanishingly low concentration because of the ubiquity of sulfhy-

dryl compounds. The same statement applies to the free valence of the mercury linked to the terminal carbon of the parent molecule; it too must be largely bound by thiols. The thesis that rupture of the carbon-mercury bond is a requisite of diuretic activity has recently been revived by Mudge and his colleagues. Mudge has observed a relationship between *in vivo* diuretic activity and *in vitro* acid lability in a series of organomercurial compounds. He explains the well recognized potentiation of mercurial diuresis by ammonium chloride in terms of the intracellular as well as extracellular acidosis which it induces. The greater the renal intracellular acidosis, the more the parent compound is broken down to diuretic mercuric ion. Inhibition of diuresis in metabolic alkalosis is presumed to result from greater stability of the parent compound. In view of Müller and Weiner's results, namely that most of the drug excreted in the urine is in the form of parent compound complexed with cysteine or acetyl cysteine, it is necessary to make the following assumption. Only a minute fraction of the drug taken up by the kidney, concentrated in the cortex, and secreted in the urine is diuretically active. The bulk of the diuretic serves no pharmacologically useful purpose; only the minute fraction which is broken down is active. In support of their thesis, Mudge et al have shown that the diuretic efficacy of Mercuhydrin (an acid-labile compound) is markedly affected by alterations in acid base balance; the efficacy of mercuric cysteine (an ionizable compound) is not. Furthermore, per mg. of mercury administered, the diuretic activity of mercuric cysteine[19] is considerably greater than that of Mercuhydrin, for all of its mercury is potentially available as mercuric ion.

While this thesis has its attractive aspects and may well be true, certain facts argue against it at the moment. The infusion of acetazoleamide and of potassium chloride, procedures which not only alkalinize the urine but presumably elevate the pH of the contents of renal tubular cells as well, do not alter the diuretic response

[19]This should not be construed as indicating that mercuric cysteine is a clinically useful diuretic, it is not, for it is highly toxic.

of the dog to Salyrgan or Neohydrin.[20] Furthermore, the induction of a marked extracellular and intracellular acidosis by the inhalation of 12 per cent $CO_2$ does not potentiate the action of mercurial diuretics, whereas a mild acidosis, induced by ammonium chloride, does. Finally, if inhibition of sulfhydryl enzymes is of significance in mercurial diuresis, it is not immediately apparent why compounds of the character of R—$Hg^+$ could not block them as well in vivo as in vitro. Mudge, however, maintains that divalent mercury is necessary to block two adjacent active sites on renal enzymes, one a sulfhydryl group, the other an amino or carboxyl group. This concept is illustrated to the left of Figure 32.

A somewhat different view has been outlined by Kessler and his colleagues, who suggested from their studies of a limited series of organomercurial compounds that steric configuration might be

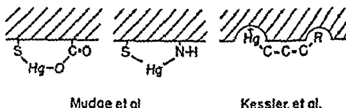



Fig. 32. Mechanism of enzyme inhibition by mercurial diuretics. Left, view of Mudge et al that the organic compound must be split to liberate divalent mercuric ion which combines with adjacent sulfhydryl and either carboxyl or amino groups. Right, view of Kessler et al that structural configuration of the organomercurial compound is significant. (From R.F. Pitts' *Am. J. Med.*, 24:745, 1958)

of greater significance in determing diuretic activity. They proposed that for the compound to form a stable complex with renal enzymes, a terminal atom of mercury must be separated by three carbons or by an equivalent inter-atomic distance from a hydrophilic group. This concept is illustrated to the right of Figure 32. Although they recognized certain exceptions in Novasurol and Cumertilin, they proposed the thesis as a working hypothesis. It should be pointed out that mercuric cysteine has a structure similar

[20] In man, the administration of acetazoleamide depresses mercurial diuresis, the administration of potassium chloride does not.

to that proposed by Kessler, if it be permissible to substitute a sulfur atom for one of the carbons of the three unit chain. It is entirely possible that inorganic mercuric ions, administered in any form, ultimately reach the kidney as mercuric cysteine. Mercuric chloride, a reasonably effective though toxic diuretic, might actually be concentrated within the kidney as mercuric cysteine, and thus fulfill the structural requisites proposed. To avoid belaboring the point, it seems best to reserve for the future a decision as to whether the parent molecule is active per se or whether splitting of the carbon mercury bond is necessary for the development of activity.

*Site of Diuretic Action and Site of Secretion of Organomercurial Compounds.* The portion of the nephron within which organomercurial compounds exert their diuretic effects has long been a subject of controversy. One approach has been the study of the pathological lesions which result from the administratoin of both inorganic and organic compounds of mercury. When minimal necrotizing doses of inorganic compounds are given, the proximal tubule and more specifically its distal portion, shows evidence of pathological change. It was pointed out earlier that succinic dehydrogenase is inhibited in this same part of the tubule by relatively massive doses of mercurial diuretics. These two observations have been cited as evidence that mercurials block reabsorption in the terminal part of the proximal tubule. However, it is well to remember that an alteration in cell structure produced by a toxic dose of a compound need not necessarily indicate the site at which a specific functional change is induced by a therapeutic dose.

In man it has been shown that mercurial diuretics in therapeutic doses reduce the capacity of the renal tubules to reabsorb glucose and to secrete para-aminohippurate. Since these functions are presumed to be localized in the proximal segment of the renal tubule, the conclusion has been drawn that mercurial diuretics block salt reabsorption in the same segment. Unfortunately for this thesis, neither the reabsorption of glucose nor the secretion of para-aminohippurate by the kidney of the dog is significantly depressed by mercurial diuretics. Mercurial diuretics do not inter-

fere with acidification of the urine or with ammonia secretion. Since both the functions are presumed to reside in the distal tubules, it has been inferred that mercurial diuretics could not exert their characteristic effects at this site. The dangers of such derived arguments are evident if one considers the fact that mercurial diuretics partially inhibit the secretion of potassium, a function no less sure in its distal localization than ammonia and acid secretion. Clearance observations have been variously interpreted as indicating proximal blockade or distal blockade of salt reabsorption, the nature of the argument depending more on the bias of the investigator than on the validity of the reasoning.

Recent studies of Kessler et al and Vander et al, utilizing the "stop-flow" method for localizing functions in the nephron of the dog, have provided objective evidence that mercurial diuretics are excreted by, and exert their major inhibitory effects on ion reabsorption in the proximal segment. Figure 33 is a summary of data illustrating these points. The "stop-flow" method, originally described by Malvin, Sullivan and Wilde, is briefly the following. One ureter of a dog is catheterized through a small flank incision and an osmotic diuresis is initiated by the infusion of 20 per cent mannitol containing creatinine and para-aminohippurate. When the urine flow from the one kidney attains a value of 8 to 10 ml. per min., the ureteral catheter is clamped for a period of 6 to 8 min. One minute before the clamp is released, a gram of inulin or ferrocyanide is given intravenously. The clamp is released and over the succeeding 3 minutes some 30 to 40 samples of roughly 1.0 ml. are collected in rapid succession.

The reasoning behind this technique is the following. On clamping the ureteral catheter, pressure builds up rapidly within the tubular system; filtration ceases or at least slows markedly. An essentially stationary column of fluid is held in contact with the tubular epithelium for 6 to 8 min. During this prolonged period of contact, the tubular epithelium performs in exaggerated fashion those operations on the static column of fluid which it normally performs in lesser degree on the moving column. When the clamp is released, urine under pressure is ejected forcibly, the first samples coming from pelvis and more distal parts of the nephron,

later samples from more proximal parts. The final samples contain increasing amounts of ferrocyanide or inulin, substances which serve to mark the time of appearance and to quantify the admixture of fresh formed glomerular filtrate. Three blood and urine samples collected immediately before and three more collected immediately after the period of clamping serve to control the procedure.

Two experiments are summarized in Figure 33: one, a control; the other, performed during a diuresis induced by the intravenous injection of 1.0 mg. $Hg^{203}$ per Kg. body weight as chlormerodrin. Plasma, control urine and the fractional urine samples were analyzed for creatinine, para-aminohippurate, sodium and, in the experiment in which chlormerodrin was given, for radiomercury as well. The urine/plasma concentration ratio for creatinine ($U/P_{Cr}$) shown at the bottom of the figure provides an indication of the site and degree of water reabsorption. $U/P_{Cr}$ is highest in the distal part of the nephron, therefore water has been reabsorbed to the greatest extent in this region.

The U/P ratios for radiomercury, for sodium and for para-aminohippurate have been divided by the simultaneous U/P ratio for creatinine. This arithmetic device accomplishes two ends. First, it corrects for variable water reabsorption in the several parts of the nephron. Second, such ratios of U/P ratios have the connotation of clearances of the substance in question divided by filtration rate: if the numerical value is less than 1.0, the substance is reabsorbed;[21] if greater than 1.0, it is secreted.

Mercury is obviously secreted most avidly in the proximal tubule; i e., the $U/P_{Hg}/U/P_{Cr}$ attains a value of 3.0 in this segment. Depression of sodium reabsorption is also most evident in the proximal tubule. Thus the $U/P_{Na}/U/P_{Cr}$ in this segment is 0.15 in the control experiment; following the diuretic, it is 0.30. The distal tubular reabsorptive mechanism which is capable of reducing urinary sodium nearly to zero seems relatively little affected by the

[21]A ratio less than 1.0 indicates that the substance is reabsorbed, providing it is freely filterable through the glomerular capillaries. However, if the substance is highly bound to plasma proteins as are mercurial diuretics, a ratio less than 1 0 is not necessarily indicative of reabsorption.

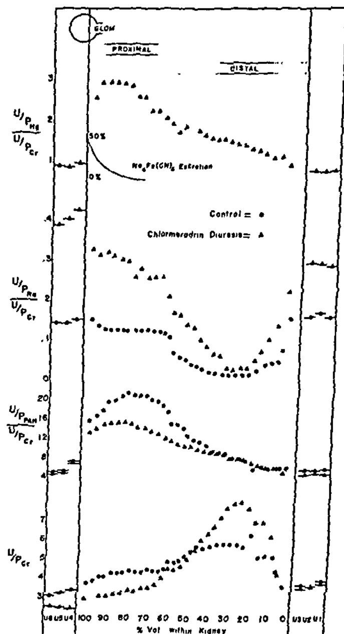



Fig. 33. "Stop-flow" experiments on the dog which localize inhibition of reabsorption of sodium by chlormerodrin and active tubular secretion of the diuretic drug to the proximal portion of the nephron. (From R.H. Kessler, K. Hierholzer, R.S. Gurd, and R.F. Pitts. *Am. J. Physiol.*, 194 540, 1958)

action of mercury. Proximal secretion of para-aminohippurate is only moderately depressed, a fact which illustrates the specificity of action of mercurials on reabsorptive transport of ions. Certainly the major action of mercurial diuretics is to reduce proximal reabsorption of sodium and chloride. Any effects which it may exert on the distal tubule are minor.

## DOSE AND ROUTE OF ADMINISTRATION

Mercurial diuretics have been administered to patients by all of the several possible routes: rectal, oral, intraperitoneal, subcutaneous, intramuscular, and intravenous.

***The Intraperitoneal Route*** has been found unsatisfactory, causing undue irritation and yielding a poor diuretic response.

***Administration by Rectal Suppository*** is occasionally effective, although the diuretic response is variable, delayed, prolonged, and generally unpredictable. At best, absorption through the rectal mucosa is poor and the dose must be correspondingly large. The most commonly used drug is Mercuhydrin, marketed in suppositories containing 190 mg. of mercury. The dose is one suppository per day inserted at bed time. Efficacy can be somewhat enhanced if an enema is given prior to insertion of the suppository. Mercuhydrin produces less rectal irritation than Salyrgan; the latter has produced ulceration. While rectal administration permits self medication, a goal worthy of achievement, the use of oral preparations is more likely to result in adequate diuresis.

***Oral Administration.*** Two preparations are especially recommended for oral use, Neohydrin and Cumertilin. Both are somewhat better absorbed by the gut than are the other diuretics. Tablets of Neohydrin contain 10 mg. of mercury; tablets of Cumertilin contain 20 mg. The dose in either instance is 1 to 4 or more tablets per day, repeated daily or in interrupted courses. Some have used Neohydrin in dosage as high as 12 tablets per day for short periods. Oral use is frequently limited by epigastric discomfort, nausea, vomiting and diarrhea. Since these difficulties are more common with larger doses, therapy should start with two tablets per day, increasing as indicated to a maximum of six to eight. If eight tablets per day are insufficient, the oral route should

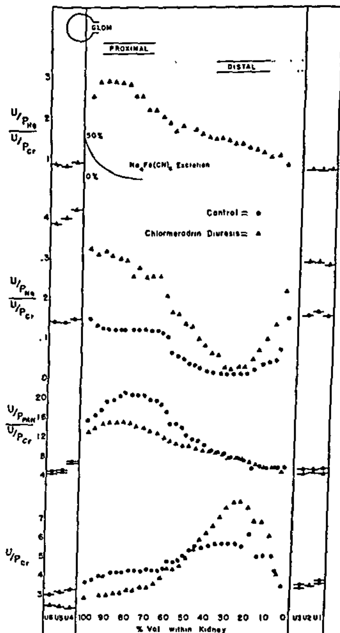



Fig. 33. "Stop-flow" experiments on the dog which localize inhibition of reabsorption of sodium by chlormerodrin and active tubular secretion of the diuretic drug to the proximal portion of the nephron. (From R.H. Kessler, K. Hierholzer, R.S. Gurd, and R.F. Pitts: *Am. J. Physiol.,* 194 540, 1958)

Thiomerin. In spite of the fact that theophylline reduces tissue reaction, intramuscular injection of most mercurial diuretics causes some burning sensation and local tenderness. Dicurin contains 45 mg. of procaine base in each ml. to minimize local discomfort. Although the local anesthetic relieves pain, it does not alter tissue reaction; hence Dicurin should not be used subcutaneously. Thiomerin and Dicurin cause least local distress; Mercuhydrin is next best tolerated. Despite these drawbacks, the intramuscular route is commonly employed. Absorption is prompt, the overall diuretic response is equal to or greater than that following intravenous injection, there is no danger of thrombosis and slough from accidental infiltration of a vein, and the acute cardiac complications of intravenous administration, rare though they may be, are avoided. *There is no circumstance which justifies intravenous administration of mercurial diuretics, even though the practice is relatively common.*

*Time Relations in Diuresis.* The time of onset and the duration of diuresis vary with the route of administration; onset is delayed and duration is longer with oral and rectal administration than with parenteral. Following 1 ml. of Mercuhydrin intramuscularly, diuresis begins within 2 hr., reaches a maximum in 4 to 6 hr. and lasts for 12 to 24 hr. It is therefore advantageous to administer the diuretic in the morning so as to avoid disturbing the patients rest at night. In young individuals the diuresis is apt to be brisk, whereas in the elderly and weak, it may be less intense and more prolonged. The loss of as much as 14 liters of edema fluid in response to a single injection has been described. This is not an especially desirable therapeutic goal, for rapid loss of fluid increases the incidence of complications. Loss of 2 to 3 lb. per day is adequate, 5 lb., the maximum sought. In normal dogs and man, the intravenous administration of mercurial diuretics initiates a diuresis in ½ hr., which reaches a peak in the second hour, and is completed in about 6 hr. It is an interesting but unexplained fact that the onset of diuresis is delayed 10 min. following the injection of a minute amount of a mercurial diuretic directly into one renal artery of a dog.

probably be abandoned, because of the high incidence of gastrointestinal symptoms with large doses. In practice, oral mercurial diuretics are frequently ineffective; they rarely produce an adequate diuretic response in the severely ill patients; and in those patients in whom they are effective, other newer oral diuretics are equally active and often better tolerated.

***Parenteral Administration.*** Of the three parenteral routes, intravenous, subcutaneous and intramuscular, the latter is the preferred. The accepted diuretics listed in Table X, all contain from 38 to 43 mg. of mercury per ml. of solution for injection. The range of therapeutic dosage in the adult is 0.5 to 2.0 ml. per day repeated as needed, i.e., weekly, semiweekly, or daily. The dose should be individualized for each patient in the same sense that the dose of digitalis is individualized. The patient in mild congestive failure should receive an initial dose of only 0.5 ml., the patient in severe failure, 1 to 2 ml., and the amount to be given subsequently should be adjusted in the light of the response to the initial dose. The smallest dose giving an adequate response (loss of 2 to 5 lb. per day) should be repeated at frequent intervals until edema fluid is fully discharged.

For children the dose should be proportionally smaller and should not exceed 1.0 mg. of mercury per Kg. per day. Several fatal reactions have been described in children, and in each instance the dose has been excessive.

De Graff has shown that the combination of the organomercurial component with equimolar quantities of theophylline greatly increases rate of absorption from the site of intramuscular injection and reduces local tissue reaction. Lehman and his associates have shown that combination with sodium thioacetate accomplishes the same end. Accordingly all diuretics recommended for parenteral use today are combined with either theophylline or thioacetate. Thiomerin, in which the organomercurial component is combined with thioacetate is the only one of the diuretics sufficiently nonirritating to be given subcutaneously. Even it has on occasion caused necrosis and sloughing of the skin. Except where the patient is to be instructed in the technique of subcutaneous self medication, the intramuscular route is advisable, even when using

overdigitalization, for they sensitize the myocardium to the actions of digitalis (see Chapter XIX).

## TOXICITY

The toxicity of organic mercurial compounds may be conveniently considered under three major headings: (1) the toxic actions of mercury in organic combination; (2) hypersensitivity of the patient to the specific diuretic administered; and (3) manifestations of toxicity to loss of water and ions.

*Toxic Actions of Organic Mercury.* Local tissue reactions to intramuscular and subcutaneous injection and to rectal and oral administration have been considered under dose and route of administration. They are mitigated but by no means eliminated by inclusion of theophylline or thioacetate in the solutions for injection. As mentioned above, Neohydrin and Cumertilin are somewhat better tolerated on oral administration, largely because they are better absorbed and hence more effective in smaller dosage than the other drugs.

The most tragic of toxic manifestations is the immediate fatal reaction. It fortunately is rare, only 30 to 40 such accidents have been described, although it is probable that more deaths have occurred than have been reported. It has occurred only on intravenous administration and for this reason, the intravenous route should never be employed. The reaction occurs immediately and consists of pallor and cyanosis, a precipitous fall in blood pressure, substernal constriction, respiratory distress, cardiac irregularity, convulsions and death in ventricular fibrillation. The vast majority of patients succumbing have shown some premonitory signs and symptoms of toxicity on previous intravenous injections. In the experimental animal, large intravenous doses of mercurial diuretics commonly produce cardiac irregularities. Combination with monothiols markedly reduces cardiotoxicity, and Thiomerin is stated to be tolerated in cats in doses up to 160 times the acute lethal dose of nonthiol-containing drugs. Nevertheless, the fact that the diuretic response to intramuscular injection is equal to or better than to intravenous injection makes it inexcusable to subject the patient to the hazard of intravenous therapy even with Thiomerin.

*Plasma Composition.* Changes in plasma composition are generally conceded to be the result, not the cause, of diuresis. Due to more rapid loss of fluid in the urine than replacement from the interstitial reservoir, the concentration of protein in the plasma, and the hematocrit and viscosity of the blood increase. For this reason profound diuresis should be avoided in the elderly and in those with any thrombotic diathesis. Coronary thrombosis and cerebral artery thrombosis have occurred following massive diuresis. However, the complication is not a common one, no doubt because the edematous patient has a large reserve of extracellular fluid, and because constriction of blood volume tends to limit the diuretic response. Chloride is commonly lost in excess of fluid and in excess of sodium so that as the plasma concentration of chloride falls, bicarbonate increases and a more or less significant metabolic alkalosis develops. Such changes attain appreciable proportions in patients subjected to intensive diuretic therapy. As mentioned earlier they progressively limit the response to successive injections and are a cause of refractoriness in some patients (vide infra.)

If the patient responds well to diuretic therapy, the loss of sodium in the urine may also exceed to some extent the loss of water. As a consequence, plasma sodium may decrease slightly, perhaps from 140 to 135 mEq. per liter, More commonly, sodium is actively conserved, and potassium and ammonia balance a significant proportion of the urinary chloride. Under these circumstances plasma potassium falls, tissue stores of potassium decline, and sodium replaces some of the intracellular potassium. The degree of depletion of potassium depends on the duration of diuretic therapy and the extent to which potassium replaces sodium in the urine. It has long been known that edematous patients, optimally digitalized, when subjected to intensive diuretic therapy, may exhibit signs of digitalis toxicity. This has in the past been explained as due to concentration of digitalis in the body fluids in consequence of the loss of water in the urine. However, loss of potassium in the urine, depletion of body stores of potassium and hypopotassemia (hypokalemia) play far more significant roles in

origin and treatment of these conditions will be considered briefly in Chapter XIX.

## POTENTIATION OF ACTION OF MERCURIAL DIURETICS

*Acidifying Agents.* It is now well recognized that the oral administration of an acidifying agent prior to the injection of a mercurial diuretic, greatly potentiates its action. Keith, Ethridge and many others have noted that ammonium chloride plus a mercurial diuretic causes a response considerably greater than the sum of the responses to the two agents given separately. Subsequently it has been observed that any agent which induces hyperchloremic metabolic acidosis potentiates the action of mercurial diuretics. Thus potentiation can be induced by the prior administration of ammonium chloride, calcium chloride, hydrogen or ammonium cycle ion exchange resins, and carbonic anhydrase inhibitors. Three explanations of potentiation have been advanced.

Mudge claims that acidosis is the significant factor, increasing the breakdown of the parent organomercurial compounds to liberate diuretically active mercuric ions in greater numbers. Increased blockade of sodium and chloride reabsorption results in enhanced urinary excretion.

Axelrod maintains that hyperchloremia is the significant factor, increasing the filtered load of chloride delivered into the renal tubules. If mercurial diuretics specifically inhibit some fraction of active reabsorption of chloride, as many believe, increasing the filtered load of chloride would cause increased urinary excretion of this ion.

The author agrees with Axelrod that hyperchloremia is the significant factor, but differs in holding that the proximal reabsorption of sodium is active whereas that of chloride is passive (see Chapter IV). In hyperchloremic acidosis, the quantity of chloride presented to the proximal tubules in the glomerular filtrate per unit time is increased relative to bicarbonate. Total sodium reabsorption remains essentially the same. Therefore, a greater than normal fraction of the sodium is reabsorbed with chloride, a lesser fraction with bicarbonate. Partial blockade of proximal sodium reabsorption by a mercurial diuretic will therefore deliver into the distal segment relatively more chloride and less bicarbonate than under

There is no doubt that large doses of mercurial diuretics in experimental animals produce signs of mercurialism not dissimilar to those produced by inorganic salts of mercury. The administration of excessive therapeutic doses, continued administration in the absence of an adequate diuretic response, administration to patients with severe renal insufficiently and marked nitrogen retention, all give rise to accumulation of drug in the body, and are potential causes of mercurialism. Manifestations of mercurialism include stomatitis, salivation, hemorrhagic colitis, and progressive renal failure. Mercurialism can be avoided if dosage is kept within a reasonable range and if the drugs are withheld in conditions known to be associated with accumulation. Many patients have received repeated injections of mercurials over periods of many years, deriving benefit from them without the development of adverse reactions.

*Reactions of Hypersensitivity.* Non-fatal and fatal toxic reactions due to an apparent anaphylactoid response to mercurial diuretics have been reported. These reactions usually occur one to two hours after administration. Asthmatic attacks and the development of acute pulmonary edema have been described. Other manifestation of drug idiosyncracy include dyspnea, substernal pain, cyanosis and circulatory collapse. Less grave signs of sensitivity include flushed skin, erythema morbilliformis, pruritis, urticaria, chills, fever, signs of bone marrow depression and exfoliative dermatitis. Changing the nature of the mercurial diuretic may ameliorate minor sensitivities. With more severe reactions, further therapy with mercurial diuretics should be avoided. Fortunately such reactions are relatively rare. Sensitization to thiomerin seems more common than to other drugs.

*Manifestations of Toxicity Secondary to Loss of Ions and Water* are by no means peculiar to mercurial diuresis. They may be encountered in therapy with any effective agent. They include acute circulatory collapse following a single profound diuresis, signs and symptoms of mild to severe hyponatremia following repeated diureses in patients maintained on a low salt regimen, and evidences of potassium depletion, including digitalis intoxication, in patients in whom dietary intake of potassium is inadequate. The

origin and treatment of these conditions will be considered briefly in Chapter XIX.

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normal conditions. Distal reabsorption of sodium can be considered as the sum of two completely independent processes: (1) the exchange of sodium ions for either hydrogen, potassium, or ammonium ions and (2) the reabsorption of sodium and chloride as ion pairs. If the mechanism responsible for process (2) is limited with respect to sodium transport capacity and essentially saturated under normal conditions, it will be swamped under conditions of mercurial diuresis and even more overwhelmed under conditions of combined mercurial diuresis and hyperchloremia. Hyperchloremia would therefore be expected to potentiate mercurial diuresis.

*Adrenal Steroids.* It has been pointed out in Chapter XII that ACTH, cortisone, hydrocortisone, prednisone, and prednisolone may induce a primary diuresis in responsive patients, but more commonly potentiate the action of mercurial diuretics and carbonic anhydrase inhibitors. Possible causes of this potentiation are discussed in the chapter on steroid therapy.

*Aminophylline.* The administration of aminophylline in the course of mercurial diuresis may significantly enhance the response. There is general agreement that this type of potentiation is due, at least in part, to increased glomerular filtration rate. Increase in the filtered load of sodium and chloride, delivered into a nephron partially inhibited by a mercurial diuretic, results in increased excretion. Aminophylline also inhibits tubular reabsorption of sodium and chloride ions and the inhibition which it induces appears to be additive to that induced by the mercurial diuretic.

*Resistance to Mercurial Diuretics.* It has long been known that certain patients, early in the course of their disease, respond well to mercurial diuretics, but as time progresses, become less and less responsive to therapy, eventually becoming completely refractory. The patient is said to be mercury-fast. For many years mercury-fastness was considered synonymous with renal tolerance to mercury, presumably developed in response to low grade renal damage repeated with each succeeding dose of diuretic. This view no doubt had its origin in the observation on experimental animals that renal tubular epithelium, regenerated following near lethal doses of bichloride of mercury, is highly resistant to further insults with the heavy metal. In opposition to this thesis are the following

facts. Many patients continue to respond in adequate fashion to mercurial diuretics over periods of many years; a few, who are severely ill, fail to respond the first time the drugs are used. Often patients who are mercury-fast can be caused to regain responsiveness by altering the therapeutic regimen. Whether true renal tolerance ever develops is debatable; other factors play greater roles in mercury resistance.

Two factors are highly significant in the development of resistance to mercurial diuretics. First, as a result of progress of the basic disease or of intercurrent infection or complication, renal merchanisms of salt conservation are stimulated to such a degree that diuretics, as previously used, are no longer effective. Second, as a result of intensive diuretic therapy, changes in volume and composition of the body fluids occur which render diuretic therapy less than normally effective. In essence both statements imply that glomerulo-tubular imbalance has increased to such a degree that a formerly effective regimen no longer produces an adequate diuretic response

A reduction in glomerular filtration rate is one of the significant causes of glomerulo-tubular imbalance leading to inadeqate diuretic response. It may be a result of progress of the primary disease process, intercurrent infection, or pulmonary embolization and infarction In the cardiac, it may be a consequence of inadequate digitalization. It may be associated with the circulatory inadequacy of the low salt syndrome. It may be a sign of potassium depletion. In any event the filtered load of sodium and chloride is so reduced that essentially all is reabsorbed, even though some portion of the transport capacity of the tubules is depressed by the diuretic. Such patients are not merely mercury-fast they are diuretic-fast and fail to respond to any agent.

The filtered load of sodium and chloride is decreased by a reduction in plasma sodium and/or chloride concentration as well as by a reduction in filtration rate. Many believe that the hypochloremia which commonly attends intensive therapy with mercurial diuretics is a significant factor, the reduction in plasma chloride concentration reducing the filtered load. Others account for the effects of intensive diuretic therapy in terms of the

metabolic alkalosis which develops. They maintain that alkalosis stablizes the carbon-mercury bond of the diuretic molecule and reduces the availability of diuretically active mercuric ions.

Glomerulo-tubular imbalance is exaggerated by increased tubular reabsorption of sodium and chloride ions no less than by reduction in filtered load. Progress of the *primary disease, infections*, and complications increase the secretion of salt retaining steroids. Inadequate digitalization, and circulatory inadequacy due to the salt depletion, dehydration and hemoconcentration which may result from diuretic therapy are potent stimuli of aldosterone secretion.

***Treatment of the Mercurial Resistant Patient.*** A variety of approaches to the therapy of edema in mercurial resistant patients is possible. One should always question the adequacy of digitalization in the patient with congestive failure who does not respond satisfactorily to mercurial diuretics despite adjuvant therapy with ammonium chloride. When increasing the dose of digitalis to the point of therapeutic response or toxicity, it is advisable to give oral potassium supplements in the form of 8 ounces of orange juice or 2 to 5 gm. of *potassium chloride* per day. Depletion of myocardial potassium may lead to arrhythmias and abnormalities of impulse conduction which may be interpreted as digitalis toxicity, even though the dosage of glycoside is less than optimum.

As pointed out above, ammonium chloride potentiates the action of mercurial diuretics and adequate dosage frequently causes the resistant patient to regain responsiveness. Ammonium chloride must be given in amounts and in a form which will induce hyperchloremic acidosis of significant proportions if it is to be effective. The required dose is 6 to 10 gm. or more per day. It should not be given in enteric coated tablets because of uncertain absorption. It should not be given in solutions more concentrated than 2.5 per cent because of gastric irritation. The daily dose should be divided equally and taken immediately before meals. A reasonable regimen is the following: 9 gm. of ammonium chloride per day for 6 days, on the fourth, fifth and sixth days, 2 ml. of a mercurial diuretic are given intramuscularly.

Some patients cannot tolerate doses of ammonium chloride adequate to produce a significant metabolic acidosis. These patients may be treated with a combination of ammonium chloride and acetazoleamide for 3 days. Because acetazoleamide interferes with mercurial diuresis, the drug is withdrawn two days prior to administration of a mercurial diuretic; the ammonium chloride is continued. On the fifth, sixth, and seventh days, 2 ml. of a mercurial diuretic are given intramuscularly. According to Luckey, this regimen is highly effective in mercurial resistant cardiacs. Ammonium chloride should not be given to patients with severe impairment of liver function, because of the danger of ammonia intoxication and hepatic coma, nor to patients with marked renal insufficiency, because of the danger of profound metabolic acidosis.

Weston has pointed out that the intravenous injection of 0.25 to 0.5 gm. of aminophylline 2 hr. after the intramuscular administration of a mercurial diuretic will frequently cause an adeqaute diuretic response in an otherwise refractory patient. Two hours is chosen to correspond to the peak diuretic effect of the mercurial. Aminophylline increases renal blood flow and filtration rate and increases the filtered load of ions delivered into the renal tubules. It also depresses renal tubular reabsorption of ions. Weston favors the oral adminstration of ammonium chloride for three days prior to combined mercurial and aminophylline therapy. He advises absolute confinement to bed for the period of diuretic action to obtain maximum benefit. The diuretic response to this regimen can be increased by elevation of the foot of the bed to a 30 degree angle and by application of elastic bandages to the edematous lower extremities from toes to thighs. This is especially useful in patients whose tissue turgor is low, namely in those who have been in failure repeatedly. The entire procedure should not be applied unless there is reason to believe that an adequate diuretic response will be obtained, because of the danger of precipitating pulmonary edema.

Others have suggested the slow intravenous infusion of as much as 4.0 ml. of a mercurial diuretic. This appears to the author to be hazardous at best, and doubly so in a patient who may well give an inadequate diuretic response. Diamox, given concurrently, tends

to reduce the diuretic response to a mercurial rather than to increase it. The actions of chlorothiazide and mercurial diuretics appear to be additive, perhaps synergistic. However, experience with combined therapy is limited.

*Contraindications to Mercurial Diuretics.* Mercurial diuretics are absolutely proscribed in acute renal failure and in acute nephritis, not only because of the danger of mercurial poisoning due to inadequate excretion, but also because of the possibility of aggravating the existing renal lesion. Some maintain that they should not be used in the nephrotic syndrome. The drugs are containdicated also in patients who have previously exhibited a toxic reaction or who have consistently failed to respond (cumulative toxicity). They may be used in chronic renal disease, if there is evidence of some renal reserve, i.e., if uremia does not develop as a consequence of dehydration.

## SUMMARY

Mercurial diuretics act directly on the renal tubules to block a fraction of the reabsorption of sodium and chloride ions. Increased urine flow and loss of body weight occur in proportion to and are the osmotic consequences of loss of ions. Mercurial diuretics are highly and specifically concentrated in the renal cortex. They probably depress ion transport by forming inactive mercaptide complexes with sulfhydryl enzymes which supply energy to drive the reabsorptive machinery. It is uncertain whether the parent molecule has diuretic properties, or decomposition to mercuric ions is a requisite of diuretic action. It is equally uncertain whether mercurial diuretics block the active reabsorption of sodium or of chloride ions, although available evidence is readily interpretable in terms of the former thesis. Mercurial diuretics are rapidly eliminated from the body following parenteral adminstration and are secreted into the urine as complexes of cysteine or acetyl cysteine. They exert their major inhibitory effects on ion transport in the proximal tubule and are themselves most actively secreted in this same segment.

Clinically, mercurial diuretics may be administered by oral or intramuscular routes. In mild salt retaining states oral administra-

tion is frequently effective; more severely ill patients require intramuscular therapy. Intravenous injection is hazardous and no more effective than intramuscular injection, it should not be employed under any circumstances.

The efficacy of diuretic therapy can be considerably enhanced by the oral administration of ammonium chloride or other acidifying agents prior to injection of a diuretic; by the administration of aminophylline at the time of expected maximum diuretic action, by confinement to bed during the phase of diuresis; and by such ancillary procedures as elevation of the foot of the bed and application of elastic bandages to assist in the return of edema fluid to the circulation. Recently it has been shown that the administration of ACTH, cortisone, prednisone, and prednisolone increase the response to mercurial diuretics in resistant patients. Treatment of intercurrent infection, correction of potassium deficiency, and in the patient with congestive failure, adequate digitalization, improve the response. Mercurial diuretics are still the most generally effective agents available for the treatment of edema.

## REFERENCES


1. Borghgraef, R. R M., Kessler, R H., and Pitts, R. F.· Plasma regression, distribution and excretion of radiomercury in relation to diuresis following the intravenous administration of Hg-203 labelled chlormerodrin to the dog *J. Clin. Invest.*, *35*:1055, 1956.
2. Kessler, R. H., Lozano, R. and Pitts, R. F. A comparison of the pharmacological behavior of chlormerodrin, meralluride, mersalyl and mercuric chloride in the dog. *J. Pharmacol. & Exper. Therap.*, *121*:432, 1957.
3. Kessler, R. H., Lozano, R. and Pitts, R. F.· Studies of structure-diuretic activity relationships of organic compounds of mercury. *J. Clin. Invest*, *36*:656, 1957.
4. Levy, R I, Weiner, I M and Mudge, G. H.. The effects of acid-base balance on diuresis produced by organic and inorganic mercurials. *J. Clin. Invest.*, *37*:1016, 1958.
5. Pitts, R. F. and Sartoris, O. W. Mechanism of action and therapeutic use of diuretics. *J. Pharmacol & Exper. Therap.* II,*98*:161, 1950.

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*Chapter XVII*

# SULFONAMYL DIURETICS

THE sulfonamyl compounds in use today as diuretics are unique therapeutic agents in that they were synthesized to block reversibly a specific renal enzyme, carbonic anhydrase. Indeed the only enzyme known to be inhibited by acetazoleamide (Diamox) and other similar unsubstituted monosulfonamyl compounds is carbonic anhydrase. Blockade of this enzyme reduces tubular reabsorption and increases urinary excretion of sodium, bicarbonate, and water. The recently introduced benzothiadiazine sulfonamyl compound, chlorothiazide (Diuril)[a] has, in addition to properties dependent on inhibition of carbonic anhydrase, the ability to depress tubular reabsorption of sodium and chloride ions. No doubt this action depends on inhibition of some other enzyme system which either serves as an ion carrier or supplies energy to a carrier mechanism. The sulfonamyl diuretics as a class are well absorbed, well tolerated, relatively non-toxic, and effective on oral administration. Accordingly, they fulfill several of the requirements of the ideal diuretic. However, they do not fulfill all, for they are frequently ineffective in the most severely ill patients. Furthermore, tolerance develops to some when they are given repeatedly, and all promote urinary loss of potassium and may deplete body reserves of this ion. However, these characteristics introduce no insurmountable difficulty in their use in properly selected patients.

The sulfonamyl compounds find their greatest use in the ambulatory patient for whom self medication is of greatest im-

[a]Hydrochlorothiazide (Hydrodiuril, Esidrix) is a recently synthesized derivative of chlorothiazide having no double bond in the heterocyclic ring. On a weight for weight basis it is claimed to be at least 5 times as potent as chlorothiazide. Whether it possesses any virtues over this latter drug as a therapeutic agent must await further tests

6. PITTS, R. F.: Some reflections on mechanisms of action of diuretics. *Am. J. Med., 24*:745, 1958.
7. RAY, C. T. and BURCH, G. E.: The mercurial diuretics. *Am. J.M. Sc. 217*:96, 1949.
8. Symposium on Mercury and its Compounds. *Ann. New York, Acad. Sc., 65*:357, 1957.
9. Symposium on Chlorothiazide and Other Diuretic Agents. *Ann. New York Acad. Sc.*, 71:321, 1958.
10. VOGL, A.: *Diuretic Therapy*, Baltimore, Williams & Wilkins, 1953, p. 248.

capillary and alveolar walls and into the alveolar gas. It is obviously advantageous to accelerate the slow dehydration reaction enzymatically. As a matter of fact, enzyme is so abundantly present in red cells that this inherently slow reaction is no longer rate limiting. Instead, the chloride-bicarbonate ion shift across the erythrocyte membrane is probably the rate limiting step in the overall transformation and translocation of plasma bicarbonate into alveolar carbon dioxide Since the reverse sequence of reactions occurs during the brief interval an erythrocyte is in a tissue capillary, the advantage of accelerating the slow hydration reaction is again obvious.

Carbonic anhydrase is a zinc containing metalloprotein enzyme having a molecular weight of about 30,000 Mann and Keilin and later Krebs showed that its activity is strongly and specifically inhibited by sulfanilamide and other $N^1$ unsubstituted sulfonamides. The sulfonamides commonly used as chemotherapeutic agents are all $N^1$ substituted sulfonamyl compounds and have no anticarbonic anhydrase activity. The reaction of enzyme and inhibitor is simply described by the equation, $\text{Enz} + \text{Inh} \rightleftharpoons \text{Enz}\cdot\text{Inh}$. The affinity of enzyme for inhibitor is great, so the reaction is driven strongly to the right. Doses of acetazoleamide used in renal research on dogs commonly interfere in some degree with pulmonary excretion of carbon dioxide, resulting in an increased $pCO_2$ in arterial blood and a mild respiratory acidosis In contrast therapeutic doses in man do not induce significant respiratory acidosis. At least one reason for this difference between dog and man is the fact that acetazoleamide is highly concentrated within the erythrocytes of the dog, but to a lesser degree in the erythrocytes of man Chlorothiazide has little or no effect on pulmonary or tissue exchanges of carbon dioxide in either man or dog, for it is not specifically concentrated in red cells.

*Carbonic Anhydrase Inhibitors as Diuretics.* Shortly after the introduction of sulfanilamide as a chemotherapeutic agent, Southworth observed that the drug induces metabolic acidosis, associated with increased urine pH and increased urinary excretion of bicarbonate. A year or so later, Mann and Keilin demonstrated the fact, referred to above, that sulfanilamide and other $N^1$ unsubstituted

portance. Because their diuretic action is less drastic than that of organomercurials, it is easier to employ them over long periods in such fashion as to maintain a stable, edema-free state. They are especially useful in those patients who, on moderate salt restriction, require occasional injections of mercurial diuretics. Such patients can take a more liberal diet when on daily doses or intermittant courses of sulfonamyl diuretics, yet maintain themselves edema-free. Occasional patients, refractory to mercurial diuretics, respond with satisfactory diureses to these agents. Although chlorothiazide has been used for a relatively short time, clinical experience to date indicates that it is a more effective and more generally useful diuretic than acetazoleamide. Sulfonamyl compounds have a place in diuretic therapy of patients with congestive failure, cirrhosis, nephrosis, nephritis, premenstrual edema, and pre-eclampsia. Relatively speaking, they are benign therapeutic agents.

*Nature of Carbonic Anhydrase.* Carbonic anhydrase was first described by Roughton as an enzyme, present in erythrocytes, which greatly accelerates the attainment of equilibrum in the reversible reaction of carbon dioxide with water to form carbonic acid, (1) $CO_2 + H_2O \rightleftarrows H_2CO_3$. The dissociation of carbonic acid to hydrogen and bicarbonate ions and the inverse association of these ions, (2) $H_2CO_3 \rightleftarrows H^+ + HCO_3$, are ionic reactions, occur instantaneously, and are uninfluenced by the enzyme. Reaction (1) in an aqueous medium at body temperature is relatively slow, requiring approximately 200 seconds to come within 10 per cent of equilibrium. Reaction (1) is greatly speeded by carbonic anhydrase; the quantity of enzyme present in red cells is sufficient theoretically to accelerate the rate of reaction in whole blood 7,500-fold.

Most of the carbon dioxide produced in metabolic reactions is transported from tissues to lungs as bicarbonate ion in the blood plasma. In the second or so required for an erythrocyte to traverse a pulmonary capillary, bicarbonate ion must diffuse into the erythrocyte in exchange for chloride ion and associate with hydrogen ion to form carbonic acid; the carbonic acid must dehydrate to carbon dioxide and water; and the carbon dioxide must diffuse out of the red cell, across the plasma stream, through the

plays an important role in making hydrogen ions available in tubular cells at a rapid rate. In confirmation of this thesis they noted that sulfanilamide markedly depresses the rate of excretion of titratable acid.

Others have adopted the view that hydrogen ions are actively secreted by a redox ion pump in which the ferric iron of the cytochrome system oxidizes hydrogen atoms to hydrogen ions; $4\ Fe^{+++} + 4H \rightleftarrows 4\ Fe^{++} + 4\ H^{+}$. The ferrous iron is then reoxidized as follows, to regenerate ferric iron and to produce equivalent numbers of hydroxyl ions, $4\ Fe^{++} + O_2 + 2\ H_2O \rightleftarrows 4\ Fe^{+++} + 4\ OH^{-}$. The role of carbonic anhydrase in such a system would be that of neutralizing hydroxyl ions; $4\ OH^{-} + 4\ CO_2 \overset{c.a.}{\rightleftarrows} 4\ HCO_3^{-}$. This system is one which has also been proposed as the mechanism of gastric secretion of acid. There is at the moment no definitive proof of either hypothesis. However, if the transfer of hydrogen ions into proximal tubular urine is passive and downhill along an electrochemical gradient as developed in Chapter IV, it is obvious that the first explanation of the role of carbonic anhydrase is correct. However, as was pointed out earlier, hydrogen ions must be actively transported in the distal tubule in exchange for sodium ions. The nature of the transport mechanism is unknown.

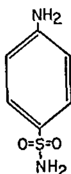
Subsequently Pitts and Lotspeich noted that sulfanilamide blocks a fraction of the reabsorption of filtered bicarbonate. They assigned this fraction to the distal tubule, pointing out that the exchange of hydrogen ions for sodium ions of a solution containing bicarbonate, would convert bicarbonate ions to carbonic acid. Dehydration of carbonic acid to $CO_2$ and water and back diffusion of $CO_2$ into renal capillary blood would be the equivalent of the reabsorption of bicarbonate ions per se. Roughly one-fifth of bicarbonate reabsorption was presumed to occur in the distal tubule by such a mechanism of ion exchange; four-fifths was presumed to occur in the proximal segment by a mechanism which specifically transports bicarbonate ion. This latter view is now known to be erroneous Proximal as well as distal bicarbonate reabsorption is probably effected by a process of $Na^{+}$ for $H^{+}$ exchange (*cf.* Figs 13, 14, 15B), but the demonstration of this fact

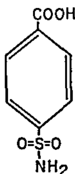
sulfonamides inhibit carbonic anhydrase activity in vitro. This, coupled with the observation of Davenport and Wilhelmi that the enzyme is highly concentrated in the renal cortex, paved the way for an explanation of the metabolic acidosis induced by sulfanilamide. Hoeber first suggested that renal carbonic anhydrase is in some way involved in the tubular reabsorption of bicarbonate, and that sulfanilamide, by inhibiting the enzyme, causes the excretion of alkaline urine and induces metabolic acidosis.

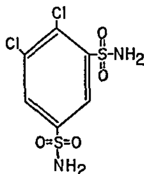
At the time two views were held as to the nature of the mechanism for acidifying the urine, both based on the premise that the acid, ultimately excreted as free titratable acid, enters the urine in the glomerular filtrate. According to one view, dibasic phosphate is preferentially reabsorbed from the tubular urine, leaving monobasic phosphate to be excreted as titratable acid. According to the other view, bicarbonate is preferentially reabsorbed, leaving carbonic acid to react with and to convert phosphate and other buffer salts into urinary titratable acid.

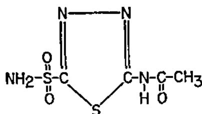
In 1945, Pitts and Alexander demonstrated that acidotic dogs, supplied with large amounts of buffer by the intravenous infusion of sodium phosphate or creatinine, excrete far more hydrogen ions in the urine than are present in the glomerular filtrate. They concluded that the renal tubular cells add hydrogen ions to the glomerular filtrate as it flows along the renal tubules. They suggested that tubular cells exchange hydrogen ions, generated within their substance by the dissociation of carbonic acid, for sodium ions in the tubular urine. The hydrogen ions and buffer anions are eliminated as titratable acid; the sodium and bicarbonate ions are reabsorbed into the peritubular capillaries to replenish the buffer reserves of the body fluids (*cf.* Fig. 15A). Since the kidneys of the dog can excrete titratable acid at a rate equivalent to 6,000 ml. of N/10 acid per day, it is obvious that an abundant source of $H^+$ ions must be available to tubular cells. Ultimately of course, the only possible source of hydrogen ions of this magnitude is water. Pitts and Alexander postulated that carbon dioxide is hydrated to carbonic acid within the acidifying cells of the distal tubule and that the carbonic acid so formed supplies hydrogen ions to the exchange mechanism. According to this view carbonic anhydrase

amino, or acylamino groups on the benzene ring increases enzyme inhibitory activity to the same order of magnitude as that of heterocyclic compounds. Dichlorphenamide (Daranide) is such a halogen substituted disulfonamyl compound. Combination of thiadiazine and benzene rings with 6 chloro, 7 sulfamyl substitution of



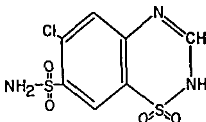




Fig 34. Structure of representative sulfonamyl diuretics.

the aromatic nucleus, as in chlorothiazide, introduces a new property, the ability to block sodium chloride reabsorption. According to Beyer, dichlorphenamide possesses this chloruretic characteristic in the same sense but to a lesser degree than chlorothiazide. Our own findings are opposed to this view. In the dog we have observed essentially no chloruresis with dichlorphenamide or acetazoleamide. Chlorothiazide, on the other hand, is very significantly chloruretic, certainly differs quantitatively and possibly qualitatively as well from the other two.

by Berliner required the development of carbonic anhydrase inhibitors several hundred times more potent than sulfanilamide.

It now became apparent to Schwartz that sulfanilamide might have potentialities as a diuretic. A study on patients in congestive failure demonstrated that this drug causes the excretion of an alkaline urine containing increased quantities of sodium, bicarbonate and potassium ions and that weight loss ensues. However, the required dosage was large and undesirable side reactions were marked. It was on the basis of these observations that Roblin and Clapp synthesized a series of highly active heterocyclic sulfonamide inhibitors of carbonic anhydrase, including acetazoleamide, in the hope of finding a clinically useful and orally effective diuretic.

## CHEMICAL NATURE OF CARBONIC ANHYDRASE INHIBITORS

A common feature of the carbonic anhydrase inhibitors employed as diuretics is the unsubstituted sulfonamyl group, $-SO_2NH_2$. However, the nature of the molecule to which this group is attached greatly affects enzyme inhibitory and diuretic properties. Roblin and Clapp observed highest enzyme inhibitory action among heterocyclic compounds such as acetazoleamide (see Fig. 34), a compound which is some 300 times as potent in vitro as sulfanilamide. *Even more potent is benzothiazole — 2 — sulfonamide*, some 800 times as active as sulfanilamide. However, in vivo it is completely devoid of diuretic properties due to rapid *metabolism and conjugation. Methazoleamide, in which a* methyl group is attached to one of the heterocyclic ring nitrogens of acetazoleamide is a highly active enzyme inhibitor, penetrates eye and brain rapidly and accordingly is a potent antiglaucoma and anticonvulsant drug. However, it possesses no virtues as a diuretic over acetazoleamide.

The aromatic monosulfonamyl compounds such as sulfanilamide and Dirnate have a relatively low order of activity. The latter, after brief exploitation as a diuretic, has been withdrawn from use. Sprague, Beyer and their associates have observed a high order of activity among aromatic disulfonamyl compounds, especially those of 1, 3 configuration. Furthermore, substitution of halogen,

suppresses a different series of reactions which furnish some 10 per cent of the energy. Since their actions are additive, the two drugs together suppress about 30 per cent of sodium and chloride transport. There remains, however, in the doubly blocked renal tubules from 60 to 70 per cent of normal ion transport capacity. The energy for this moiety is presumably supplied by metabolic reactions not sensitive either to mercurial diuretics or chlorothiazide.

## RENAL ACTIONS OF SULFONAMYL DIURETICS

*Acetazoleamide.* Maren has described the diuretic effects of acetazoleamide in normal dogs in the following terms. *When the drug is given in a single daily dose of 5 to 10 mg. per Kg., it* promptly alkalinizes the urine. The excretion of sodium, potassium, and bicarbonate is increased, that of ammonia and titratable acid is sharply reduced. The duration of action is relatively short (6 hr. or less), and during the remainder of the day the losses of ions are made up from dietary intake, and the acidosis which results from bicarbonate loss is corrected by enhanced ammonia and titratable *acid excretion. Under such conditions, the drug exerts a more or* less constant action from day to day; i.e., tolerance does not develop. However, if dosage is increased and especially if multiple doses are administered, initial ion losses are greater, effects are more prolonged, and after a period, the animal becomes refractory to the drug.

Table XIII summarizes an experiment of Pitts *et al.* which illustrates the acute response of a dog to a relatively large dose of acetazoleamide administered intravenously. The animal was prehydrated with saline in order that the diuresis might not be curtailed by depletion of extracellular fluid reserves. The first two collection periods describe control observations; the succeeding four, describe observations made immediately following the intravenous administration of 10 mg. per Kg. of acetazoleamide as a priming dose and the addition of drug to the infusion in amounts sufficient to provide 15 mg. per Kg. per hr. Little consideration should be accorded urine flow in such experiments as these, for it is the least reliable index of diuretic activity.

*Enzyme Inhibitory Actions of Sulfonamyl Compounds.* Th heterocyclic and aromatic monosulfonamyl compounds and th aromatic disulfonamyl compound, dichlorphenamide, exhibit major, if not a single, enzyme inhibitory action, namely, that c blocking carbonic anhydrase. The concept of Roblin that enzym inhibition results from steric similarities between the sulfonamy group and carbonic acid is illustrated in Figure 35. The inhibito

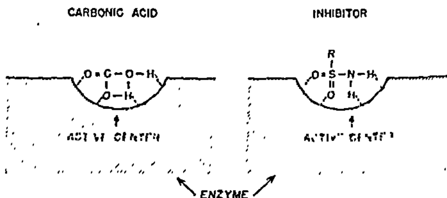



Fig. 35. Concept of Dr. R O Roblin of the mechanism of action of carbonic anhydrase inhibitors based on steric similarities between carbonic acid and the unsubstituted sulfonamyl group. (From J. M Sprague· *Ann. New York Acad. Sc.*, 71:321, 1958.)

has a stronger affinity for the active sites on the enzyme molecule than does carbonic acid so that the later is displaced and enzymatic activity abolished.

Chlorothiazide probably possesses an enzyme inhibitory action in addition to that of blocking carbonic anhydrase. The nature of the enzyme affected is unknown, except that it is concerned with the renal tubular reabsorption of sodium and chloride. Chlorothiazide blocks sodium chloride transport to a significant degree, but whether by interfering with a carrier mechanism or with some metabolic process which supplies energy to a carrier mechanism is uncertain. Pitts and his associates favor this latter view. They suggest that chlorothiazide, like organomercurial compounds, interferes with the sodium pump of proximal tubular cells. Mercurials suppress metabolic reactions which furnish roughly 20 per cent of the energy required to drive the pump; chlorothiazide

It is apparent that the drug reduced plasma pH and increased urine pH within the first 10 minutes after the start of intravenous infusion. The decrease in plasma pH resulted from an increase in the $pCO_2$ of arterial blood due to interference with liberation of $CO_2$ in the lungs. The increase in urine pH resulted from a marked increase in excretion of bicarbonate, from control values of 32 and 17 $\mu$Eq. per min. to well over 400 $\mu$Eq. per min. Increased excretion of bicarbonate was due to depression of tubular reabsorption from 99 per cent of that filtered to 64 per cent. The increased urinary bicarbonate was balanced in part by sodium and in part by potassium. Chloride excretion was little changed either in terms of absolute rate of excretion or in terms of per cent of the filtered moiety reabsorbed.

It should be remembered that potassium is both reabsorbed from and secreted into the tubular urine. Apparent depression of tubular reabsorption from some 90 per cent to 14 to 40 per cent may actually represent an increase in tubular secretion. Berliner believes that reabsorption of potassium is nearly complete in the proximal tubule, and that variations in excretion, such as those induced by acetazoleamide, are in truth variations in tubular secretion of potassium in the distal segment.

In the experiment summarized in Table XIII, the dog was moderately acidotic in the control periods (plasma pH 7.3; plasma $BHCO_3$, 18 mEq. per liter). This acidosis, metabolic in nature, was dilutional in origin, and due to the large amount of saline administered prior to the start of the experiment. Had the plasma bicarbonate been more within the normal range (24 to 28 mEq. per liter), urinary losses of bicarbonate would probably have been greater.

***Dichlorphenamide*** *is in many respects similar in its renal actions* to acetazoleamide, depressing reabsorption and increasing urinary excretion of sodium, potassium and bicarbonate ions. Due to increased excretion of bicarbonate, the urine becomes alkaline. According to Beyer, dichlorphenamide exerts a more prolonged and profound depression of ion reabsorption at comparable dose levels, is more active under conditions of low salt intake, and is less subject to the development of tolerance than acetazoleamide.

TABLE XIII

THE EFFECTS OF A LARGE DOSE OF ACETAZOLEAMIDE ON REABSORPTION AND EXCRETION OF IONS BY THE DOG

| Total Elapsed Time | Urine Flow | Glom. Filt. Rate | pH | | Plasma Concentration | | | | Rate of Excretion | | | | Per Cent of Filtered Reabsorbed | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Plasma | Urine | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ |
| (min) | (ml. per min) | | | | (milliequivalents per liter) | | | | (microequivalents per minute) | | | | | | | |
| *Dog wt. = 21 Kg.* | | | | | | | | | | | | | | | | |
| 0-10 | 3.9 | 109 | 7.30 | 6.76 | 149 | 3.50 | 123 | 18.5 | 882 | 23 | 843 | 32 | 94.3 | 93.8 | 94.0 | 98.7 |
| 10-20 | 4.2 | 98.4 | 7.32 | 6.50 | 150 | 3.66 | 124 | 18.1 | 808 | 29 | 731 | 17 | 94.3 | 91.5 | 94.3 | 99.0 |
| 20 *Acetazoleamide: 10 mg. per Kg., Prime; 15 mg. per Kg. per hr., Infusion* | | | | | | | | | | | | | | | | |
| 20-30 | 5.0 | 82.9 | 7.20 | 7.58 | 150 | 3.39 | 122 | 16.0 | 980 | 230 | 720 | 445 | 91.8 | 18.1 | 93.1 | 68.0 |
| 30-40 | 5.5 | 86.0 | 7.22 | 7.59 | 150 | 3.22 | 122 | 15.2 | 1150 | 142 | 736 | 488 | 90.7 | 46.0 | 93.3 | 64.3 |
| 40-50 | 5.2 | 76.4 | 7.21 | 7.56 | 148 | 3.24 | 125 | 16.3 | 1060 | 130 | 655 | 468 | 90.0 | 44.7 | 93.4 | 64.2 |
| 0-60 | 4.2 | 78.7 | 7.22 | 7.61 | 147 | 3.04 | 123 | 16.0 | 800 | 160 | 679 | 434 | 92.6 | 29.5 | 93.3 | 67.1 |

(From R. F. Pitts, F. Krück, R. Lozano, D. W. Taylor, O. P. A. Heidenreich, and R. H. Kessler: *J. Pharmacol. & Exper. Therap.*, 123:89, 1958.)

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Plasma | Urine | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ |
| (min.) | (ml. per min.) | | | | (milliequivalents per liter) | | | | (microequivalents per minute) | | | | | | | |
| | | | | | | | | Dog wt. = 21 Kg. | | | | | | | | |
| 0-10 | 3.9 | 109 | 7.30 | 6.76 | 149 | 3.50 | 123 | 18.5 | 882 | 23 | 843 | 32 | 94.3 | 93.8 | 94.0 | 98.7 |
| 10-20 | 4.2 | 98.4 | 7.32 | 6.50 | 150 | 3.66 | 124 | 18.1 | 808 | 29 | 731 | 17 | 94.3 | 91.5 | 94.3 | 99.0 |
| 20 | Acetazoleamide: 10 mg. per Kg., Prime; 15 mg. per Kg. per hr., Infusion | | | | | | | | | | | | | | | |
| 20-30 | 5.0 | 82.9 | 7.20 | 7.58 | 150 | 3.39 | 122 | 16.0 | 980 | 230 | 720 | 445 | 91.8 | 18.1 | 93.1 | 68.0 |
| 30-40 | 5.5 | 86.0 | 7.22 | 7.59 | 150 | 3.22 | 122 | 15.2 | 1150 | 142 | 736 | 488 | 90.7 | 46.0 | 93.3 | 64.3 |
| 40-50 | 5.2 | 76.4 | 7.21 | 7.56 | 148 | 3.24 | 125 | 16.3 | 1060 | 130 | 655 | 468 | 90.0 | 44.7 | 93.4 | 64.2 |
| 50-60 | 4.2 | 78.7 | 7.22 | 7.61 | 147 | 3.04 | 123 | 16.0 | 800 | 160 | 679 | 434 | 92.6 | 29.5 | 93.3 | 67.1 |

(From R. F. Pitts, F. Kruck, R. Lozano, D. W. Taylor, O. P. A. Heidenreich, and R. H. Kessler: *J. Pharmacol. & Exper. Therap.*, 123:89, 1958.)

It is apparent that the drug reduced plasma pH and increased urine pH within the first 10 minutes after the start of intravenous infusion. The decrease in plasma pH resulted from an increase in the $pCO_2$ of arterial blood due to interference with liberation of $CO_2$ in the lungs. The increase in urine pH resulted from a marked increase in excretion of bicarbonate, from control values of 32 and 17 $\mu$Eq. per min. to well over 400 $\mu$Eq. per min. Increased excretion of bicarbonate was due to depression of tubular reabsorption from 99 per cent of that filtered to 64 per cent. The increased urinary bicarbonate was balanced in part by sodium and in part by potassium. Chloride excretion was little changed either in terms of absolute rate of excretion or in terms of per cent of the filtered moiety reabsorbed.

It should be remembered that potassium is both reabsorbed from and secreted into the tubular urine. Apparent depression of tubular reabsorption from some 90 per cent to 14 to 40 per cent may actually represent an increase in tubular secretion. Berliner believes that reabsorption of potassium is nearly complete in the proximal tubule, and that variations in excretion, such as those induced by acetazoleamide, are in truth variations in tubular secretion of potassium in the distal segment.

In the experiment summarized in Table XIII, the dog was moderately acidotic in the control periods (plasma pH 7.3; plasma $BHCO_3$, 18 mEq. per liter). This acidosis, metabolic in nature, was dilutional in origin, and due to the large amount of saline administered prior to the start of the experiment. Had the plasma bicarbonate been more within the normal range (24 to 28 mEq. per liter), urinary losses of bicarbonate would probably have been greater.

*Dichlorphenamide* is in many respects similar in its renal actions to acetazoleamide, depressing reabsorption and increasing urinary excretion of sodium, potassium and bicarbonate ions. Due to increased excretion of bicarbonate, the urine becomes alkaline. According to Beyer, dichlorphenamide exerts a more prolonged and profound depression of ion reabsorption at comparable dose levels, is more active under conditions of low salt intake, and is less subject to the development of tolerance than acetazoleamide.

## TABLE XIV

THE EFFECTS OF A LARGE DOSE OF CHLOROTHIAZIDE ON REABSORPTION AND EXCRETION OF IONS BY THE DOG

| Total lapsed Time | Urine Flow | Glom. Filt. Rate | pH | | Plasma Concentration | | | | Rate of Excretion | | | | Per Cent of Filtered Reabsorbed | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Plasma | Urine | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ |
| (min) | (ml per min) | | | | (milliequivalents per liter) | | | | (microequivalents per minute) | | | | | | | |
| Dog. wt. = 18 Kg. | | | | | | | | | | | | | | | | |
| 0-10 | 4.7 | 91.7 | 7.30 | 6.06 | 146 | 3.75 | 122 | 19.2 | 560 | 25 | 590 | 5 | 95.5 | 92.5 | 95.0 | 99.8 |
| 10-20 | 3.9 | 92.1 | 7.33 | 5.98 | 147 | 3.64 | 120 | 18.4 | 550 | 26 | 560 | 3 | 95.8 | 91.8 | 95.0 | 99.9 |
| 20 Chlorothiazide: 10 mg. per Kg., Prime; 15 mg. per Kg per hr., Infusion | | | | | | | | | | | | | | | | |
| 20-30 | 7.1 | 84.2 | 7.33 | 7.04 | 147 | 3.75 | 121 | 17.9 | 1120 | 68 | 1020 | 160 | 90.5 | 77.3 | 90.4 | 90.0 |
| 30-40 | 8.2 | 82.6 | 7.33 | 7.27 | 147 | 3.64 | 120 | 17.8 | 1290 | 147 | 1150 | 204 | 88.8 | 49.3 | 88.9 | 86.8 |
| 40-50 | 7.2 | 75.9 | 7.32 | 7.26 | 147 | 3.63 | 124 | 17.7 | 1090 | 126 | 1010 | 160 | 89.6 | 51.9 | 89.8 | 88.7 |
| 50-60 | 6.7 | 75.5 | 7.30 | 7.23 | 148 | 3.63 | 122 | 17.9 | 1070 | 111 | 1010 | 143 | 89.9 | 57.4 | 89.5 | 90.0 |

(From R. F. Pitts, F. Kruck, R. Lozano, D. W. Taylor, O. P. A. Heidenreich, and R. H. Kessler: *J. Pharmacol. & Therap.* 123:89, 1958)

Since the two drugs inhibit carbonic anhydrase in vitro to nearly the same degree, greater in vivo activity of dichlorphenamide might result from greater concentration in, or more ready penetration of renal tissue. Such properties have not been demonstrated. It is claimed that dichlorphenamide depresses reabsorption and increases excretion of chloride to a significantly greater extent than does acetazoleamide.

*Chlorothiazide.* Beyer and his associates have shown that chlorothiazide exhibits properties both of carbonic anhydrase inhibitors and of mercurial diuretics. Due to the free sulfonamyl group, it inhibits carbonic anhydrase in vitro, and in relatively large doses in vivo, alkalinizes the urine, depresses the reabsorption of bicarbonate, and promotes the secretion of potassium. In addition it causes natriuresis and chloruresis, actions similar to those of mercurial diuretics. In fact in the usual orally effective doses, the major response is natriureses and chloruresis. The urine may fail to become alkaline, although potassium excretion is enhanced. However, the basic mechanisms of chloruretic action of chlorothiazide and of the organomercurial compounds differ in the sense that their actions are additive when the two are given together, each in maximally effective dosage. Furthermore, BAL (dithiopropanol) blocks the action of mercurial diuretics, whereas it has no effect on chlorothiazide While chlorothiazide can induce diuresis in the presence of fluid retention induced by corticosteroids, it is not a competitive antagonist in the sense of the antialdosterones, for it enhances rather than reduces the potassium loss characteristically induced by these steroids.

Table XIV summarizes an experiment of Pitts et al which illustrates the acute response of a dog to a relatively large dose of chlorothiazide administered intravenously. The animal was prehydrated in exactly the same manner employed in the experiment summarized in Table XIII. The two experiments are, therefore, as nearly comparable as any two are likely to be on different mongrel dogs.

It is apparent that the drug increased urine pH within the first 10 mins. after intravenous administration, yet had no discernable effect on plasma pH. The increase in urine pH resulted from a

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Plasma | Urine | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ | Na | K | Cl | $HCO_3$ |
| (min) | (ml. per min) | | | | (milliequivalents per liter) | | | | (microequivalents per minute) | | | | | | | |
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It is apparent that the drug increased urine pH within the first 10 mins. after intravenous administration, yet had no discernable effect on plasma pH. The increase in urine pH resulted from a

significant increase in excretion of bicarbonate, from control values of 5 and 3 μEq. per min. to as much as 200 μEq. per min. Even more striking was the chloruresis induced by the drug, the rate of excretion of chloride increasing from 560 μEq. per min. to over 1000 μEq. per min. The increase in excretion of chloride was roughly balanced by an equivalent increase in excretion of sodium. Both chloruresis and natriuresis resulted from depression in tubular reabsorption, from 95 per cent of that filtered to 89 per cent. Reabsorption of potasisum was diminished and excretion enhanced. The same reservations with respect to the meaning of diminished potassium reabsorption, as were outlined above for acetazoleamide, apply to chlorothiazide. Again the moderate dilutional metabolic acidosis reduced in some degree the extent of bicarbonate excretion in comparison with that which would have obtained had the plasma level been within a more normal range.

*Comparison of Renal Actions of Acetazoleamide, Dichlorphenamide, and Chlorothiazide.* In vitro, acetazoleamide and dichlorphenamide are significantly more potent inhibitors of carbonic anhydrase than is chlorothiazide. This is reflected in a more pronounced inhibition of reabsorption and a greater increase in excretion of bicarbonate following acetazoleamide and dichlorphenamide than chlorothiazide. A somewhat different but related expression of carbonic anhydrase inhibition is promotion of urinary potassium loss. Again the activity of acetazoleamide and dichlorphenamide exceeds that of chlorothiazide. Still a third expression of this property is depression of blood pH due to interference with $CO_2$ elimination in the lungs. Acetazoleamide is the most active of the drugs in this respect. Chlorothiazide possesses a property which distinguishes it quantitatively and probably qualitatively as well from acetazoleamide and dichlorphenamide, namely, the ability to block the reabsorption of sodium and chloride ions.

From the above noted considerations, one would predict that chlorothiazide would be a more effective diuretic for clinical use than either acetazoleamide or dichlorphenamide, i.e., it would cause less disturbance in electrolyte pattern of the body fluids, it would cause less depletion of body stores of potassium, and it would cause greater loss of sodium and chloride ions All three

drugs depress glomerular filtration rate when administered intravenously in large doses. However, when given orally in the usual therapeutic doses, they have no significant effect on glomerular function.

## SITE OF ACTION OF ACETAZOLEAMIDE, DICHLORPHENAMIDE, AND CHLOROTHIAZIDE

*Acetazoleamide.* If one neglects depression of glomerular filtration rate which commonly results when large doses of acetazoleamide are given intravenously, all renal actions of this drug can be explained in terms of a specific depression of mechanisms for exchange of hydrogen ions for sodium ions. This action, exerted on proximal tubules, reduces reabsorption of bicarbonate (*cf.* Fig. 13). Exerted on distal tubules, it further reduces bicarbonate reabsorption and inhibits acidification of the urine (*cf.* Fig. 15 A,B). Since the urine becomes alkaline, the distal secretion of ammonia is reduced (*cf.* Fig. 15C). The mechanism for hydrogen and sodium exchange is also concerned with potassium and sodium exchange. The overall activity of this joint mechanism in reabsorbing sodium is only moderately depressed by acetazoleamide. Accordingly, blockade of hydrogen exchange by the drug facilitates potassium exchange, and exhibition of acetazoleamide in large doses frequently causes net potassium secretion. The drug has little effect on other renal transport mechanisms, such as reabsorption of glucose and secretion of para-aminohippurate.

Figure 36 illustrates both the nature and the localization within the nephron of these actions of acetazoleamide. These data were derived from an experiment on a dog utilizing the "stop flow" method of localizing tubular functions described in Chapter XVI. The experiment was performed in two parts, the first consisted of a control series of observations, the second consisted of a series following the administration of 10 mg. per Kg. of acetazoleamide as a priming dose and the addition of the drug to the infusion in amounts sufficient to provide 15 mg. per Kg. per hr. In each of the two series of observations the ureteral catheter was clamped for a period of four minutes.

If one surveys Figure 36 from top to bottom, the significant

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If one surveys Figure 36 from top to bottom, the significant

actions of acetazoleamide are readily apparent. The control experiment is identified by circles, the acetazoleamide experiment by triangles. Urine samples obtained from the distal part of the nephron, i.e. those to the right of the graph, were acidified from a baseline value of pH 8.0[23] to roughly pH 5.0 in the control experiment. Following acetazoleamide, distal acidification was blocked. In the control experiment, ammonia was secreted into the distal urine samples, in highest concentration at the site of maximum acidification. Following acetazoleamide, and as a direct consequence of failure of acidification, ammonia secretion was inhibited. In contrast, the distal secretion of potassium, relatively slight in the control experiment, was greatly exaggerated by acetazoleamide.[24] Distal reabsorption of sodium was relatively unaffected by the drug.

This experiment graphically portrays the reciprocal relationship between the exchange of hydrogen and ammonium ions or potassium ions for sodium, and illustrates the fact that a carbonic anhydrase inhibitor, by depressing distal hydrogen and ammonia exchange, facilitates potassium exchange. It should be pointed out that distal reabsorption of sodium ($U/P_{Na}/U/P_{Cr} < 0.05$) can by no means be ascribed solely to potassium, hydrogen and ammonia exchange. Simple calculations indicate that most of the sodium is reabsorbed in the distal segment in association with chloride ions Only a small, albeit significant, fraction of the total is exchanged for $H^+$, $K^+$ and $NH_4^+$.Furthermore, it is apparent that the site of most avid reabsorption of sodium is slightly more proximal than

[23]Since it was impossible with the experimental techniques employed to prevent loss of $CO_2$, all specimens were equilibrated with air prior to measurement of pH. This exaggerates pH differences, for it causes those samples which contain bicarbonate (proximal) to become more alkaline, whereas those with negligible bicarbonate (distal) do not change reaction.

[24]The high $U/P_K/U/P_{Cr}$ values in the proximal tubule cannot be interpreted with any degree of assurance. The ratio of 1.5 to 2.0 might be considered as indicating potassium secretion in the proximal segment. However, samples held in contact with proximal epithelium for the 4 min. period of clamping must pass through the distal segment en route to collection. It is, therefore, probable that much of the potassium in these samples was added during transit through the distal part of the nephron Any attempt to quantify a proximal contribution to potassium excretion is so dependent on assumptions as to render it of doubtful value.

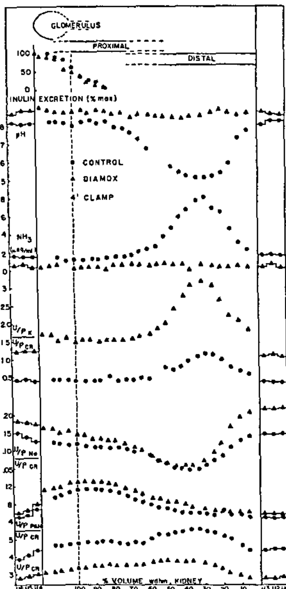



Fig. 36. "Stop-flow" experiments on a dog which localize inhibition of urine acidification and of ammonia secretion and stimulation of potassium secretion by acetazoleamide to the distal nephron The data indicate equivocal depression of proximal sodium reabsorption and negligible depression of distal sodium reabsorption. See Chapter XVI, page 232, for description of stop flow method. (From R. F. Pitts, R. S Gurd, R. H. Kessler, and K. Hierholzer *Am J. Physiol*, *194*:125, 1958)

the site of maximum exchange of sodium for hydrogen, ammonia, and potasisum.

One additional finding apparent in Figure 36 possibly has significance. Values of $U/P_{Na}/U/P_{Cr}$ in specimens from the proximal segment are uniformly higher after acetazoleamide than in control experiments; i.e., proximal reabsorption of sodium is inhibited to some extent by the drug. It is probable that the inhibited fraction is that ordinarily reabsorbed in exchange for hydrogen and thus represents sodium bicarbonate. The effect is small because sodium bicarbonate reabsorption accounts for no more than one-fifth of total proximal sodium reabsorption and the dose of acetazoleamide is by no means adequate to block all.

*Dichlorphenamide* produces the same alterations in renal function at the same sites within the nephron as does acetazoleamide. Figure 37 describes a control experiment and an experiment performed immediately after the administration of dichlorphenamide. Both the control and dichlorphenamide experiments were performed on the same kidney of the same dog. It is evident that dichlorphenamide blocks ammonia secretion and acidification of the urine, and enhances the secretion of potassium, all functions of the distal portion of the nephron. The drug has little effect on sodium or chloride reabsorption in either the proximal or distal nephron. It is impossible in experiments such as these to distinguish between the actions of dichlorphenamide and acetezoleamide, if indeed any differences exist.

*Chlorothiazide.* Figure 38 illustrates both the nature and localization within the nephron of the actions of chlorothiazide. The data were derived from an experiment identical with those of Figures 36 and 37, except that following the control series of observations, chlorothiazide was administered intravenously in the same dosage employed for acetazoleamide. Since the dose of chlorothiazide was large in comparison with that administered clinically, the qualitative and even quantitative manifestations of carbonic anhydrase inhibition are essentially the same as those following acetazoleamide and dichlorphenamide. Thus depression of ammonia secretion, depression of urine acidification, and enhancement of potassium secretion in the distal nephron are essen-

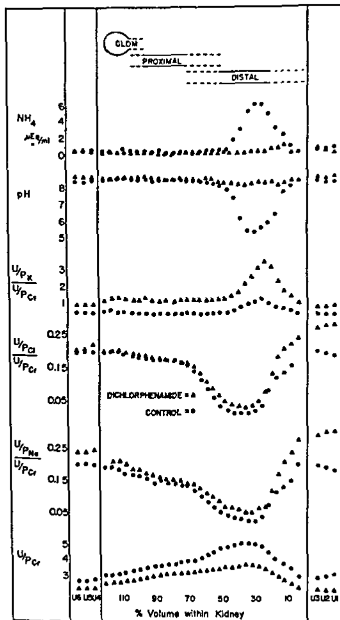



Fig 37. "Stop-flow" experiments on a dog which localize inhibition of urine acidification and of ammonia secretion and stimulat...

Pitts- *Am. J. Physiol.*, 196:1346, 1959.

tially as complete following chlorothiazide as acetazoleamide or dichlorphenamide. The distal reabsorption of sodium and especially the distal reabsorption of chloride are little if at all affected by chlorothiazide. In this respect also chlorothiazide is similar to acetazoleamide and dichlorphenamide. However, proximal reabsorption of sodium is much more significantly depressed by chlorothiazide than by the other two diuretics. Furthermore, it is evident that proximal depression of sodium reabsorption is associated with a nearly equivalent depression of chloride reabsorption. Proximal reabsorption of bicarbonate may well be depressed by chlorothiazide but is impossible to identify this action because of the greater effect on chloride. It is obvious that chlorothiazide is secreted by the proximal tubules, the $U/P_{Clu}/U/P_{Cr}$ ratio attaining values as high as 11.0. The mechanism which secretes chlorothiazide is the same as that which secretes paraaminohippurate, penicillin, diodrast, phenol red, etc.

*Absorption, Distribution, and Excretion.* According to Maren, doses of acetazoleamide within the therapeutic range are completely absorbed from the gastrointestinal tract of the dog within 2 hr. Some 70 per cent of the administered dose is excreted in the urine as active drug within 24 hr., the greater proportion within the first 6 hr. In man, over 90 per cent of the dose is excreted in the urine. Acetazoleamide is distributed in a volume equivalent to 40 per cent of body weight in the dog; in a slightly smaller volume in man. It penetrates intraoccular fluid and cerebrospinal fluid (transcellular fluids) but distributes in a concentration considerably lower than that of plasma. It is filtered through the glomeruli and partially reabsorbed by the renal tubules, the clearance of acetazoleamide averaging about two-thirds that of the simultaneously determined creatinine clearance The drug is somewhat concentrated in kidney, pancreas and erythrocytes. When given in single daily doses, the plasma concentration drops each day to indeterminate levels after 6 hr.; the red cell concentration, in contrast, plateaus at such a level as to interfere in some degree throughout the day with carbon dioxide transport in lungs and tissues (in the dog but not in man).

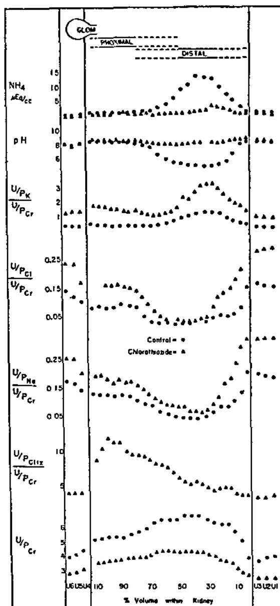



Fig 38 "Stop-flow" experiments on a dog which localize inhibition of urine acidification and of ammonia secretion and stimulation of potassium secretion by chlorothiazide to the distal nephron. The data indicate significant depression of reabsorption of sodium and chloride ions in the proximal nephron (From R. H. Kessler, K. Hierholzer, R S. Gurd, and R. F. Pitts· *Am. J. Physiol*, *196*·1346, 1959.

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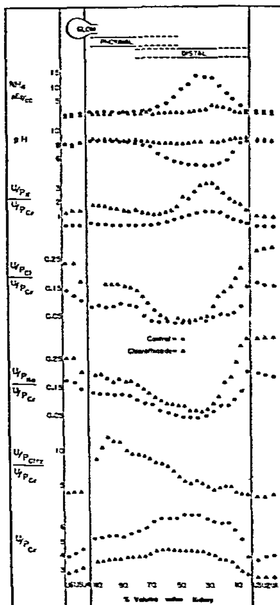



Fig. 38. "Stop-flow" experiments on a dog which localize inhibition of urine acidification and of ammonia secretion and stimulation of potassium secretion by chlorothiazide to the distal nephron. The data indicate significant depression of reabsorption of sodium and chloride ions in the proximal nephron. (From R. H. Kessler, K. Hierholzer, R. S. Gurd, and R. F. Pitts: *Am. J. Physiol.*, 196:1346, 1959.)

tially as complete following chlorothiazide as acetazoleamide or dichlorphenamide. The distal reabsorption of sodium and especially the distal reabsorption of chloride are little if at all affected by chlorothiazide. In this respect also chlorothiazide is similar to acetazoleamide and dichlorphenamide. However, proximal reabsorption of sodium is much more significantly depressed by chlorothiazide than by the other two diuretics. Furthermore, it is evident that proximal depression of sodium reabsorption is associated with a nearly equivalent depression of chloride reabsorption. Proximal reabsorption of bicarbonate may well be depressed by chlorothiazide but is impossible to identify this action because of the greater effect on chloride. It is obvious that chlorothiazide is secreted by the proximal tubules, the $U/P_{Clz}/U/P_{Cr}$ ratio attaining values as high as 11.0. The mechanism which secretes chlorothiazide is the same as that which secretes paraaminohippurate, penicillin, diodrast, phenol red, etc.

*Absorption, Distribution, and Excretion.* According to Maren, doses of acetazoleamide within the therapeutic range are completely absorbed from the gastrointestinal tract of the dog within 2 hr. Some 70 per cent of the administered dose is excreted in the urine as active drug within 24 hr., the greater proportion within the first 6 hr. In man, over 90 per cent of the dose is excreted in the urine. Acetazoleamide is distributed in a volume equivalent to 40 per cent of body weight in the dog; in a slightly smaller volume in man. It penetrates intraoccular fluid and cerebrospinal fluid (transcellular fluids) but distributes in a concentration considerably lower than that of plasma. It is filtered through the glomeruli and partially reabsorbed by the renal tubules, the clearance of acetazoleamide averaging about two-thirds that of the simultaneously determined creatinine clearance. The drug is somewhat concentrated in kidney, pancreas and erythrocytes. When given in single daily doses, the plasma concentration drops each day to indeterminate levels after 6 hr.; the red cell concentration, in contrast, plateaus at such a level as to interfere in some degree throughout the day with carbon dioxide transport in lungs and tissues (in the dog but not in man).

is exchanged for sodium and drained from tissues. Hypopotassemia and depletion of cellular stores of potassium result.

The extent of the changes in plasma and tissue composition depends on the magnitude and frequency of the dose, on the nature of the drug administered and on the adequacy of dietary intake of ions. Since acetazoleamide and dichlorphenamide are more powerful inhibitors of carbonic anhydrase than is chlorothiazide, significant aberrations in body fluid composition are more prone to follow their use. However, significant potassium depletion occurs when chlorothiazide is administered in repeated doses if dietary intake is inadeqaute. Acidosis is less a problem with chlorothiazide than with acetazoleamide and dichlorphenamide. In fact certain patients develop a mild hypochloremic alkalosis while on continued therapy with chlorothiazide due to perferential loss of sodium and chloride ions.

*Drug Refractoriness* with respect to effects on excretion of sodium and bicarbonate ions derives in part from the above mentioned changes in plasma composition. Metabolic acidosis, resulting from urinary loss of bicarbonate, reduces the filtered load of bicarbonate delivered into the renal tubules. Even though availability of hydrogen ions to the proximal and distal exchange mechanisms is reduced by carbonic anhydrase inhibitors, the uncatalyzed rate of hydration of carbon dioxide to carbonic acid may be adequate to provide for the complete reabsorption of a reduced amount of filtered bicarbonate. Accordingly, depression of reabsorption of sodium bicarbonate is self-limited. It is claimed that development of refractoriness to dichlorphenamide is much less a limiting factor in its use as a diuretic than is that to acetazoleamide. Why this should be is not clear to the author. Since the major therapeutic effect of chlorothiazide is depression of sodium and chloride reabsorption, refractoriness in the sense described does not occur.

Enhanced excretion of potassium is considerably less self limited, and when sulfonamyl compounds are administered daily, potassium loss may continue, even though sodium and bicarbonate excretion are sharply curtailed and the urine remains acid. If dietary intake of potassium is inadequate as a consequence of

Little is known concerning rate of absorption, volume of distribution and mechanism of excretion of dichlorphenamide, for no chemical methods are available to quantify the drug in blood, urine, or tissue. However, dichlorphenamide must be rapidly absorbed in the gut, for diuresis begins within an hour after its oral administration. Since its action is more prolonged than that of acetazoleamide, it is likely that its rate of absorption or rate of excretion is lower.

According to Beyer, chlorothiazide is rapidly absorbed from the gut. It appears in the blood stream in determinable amounts within 15 min. after an oral dose, reaches peak concentrations within 45 min., and declines over 6 hr. or more. On an average, 50 per cent of an oral dose is excreted in 6 hr.; over 95 per cent of an intravenous dose is eliminated in the same period of time. The renal clearance of chlorothiazide is high, approximating effective renal plasma flow. As pointed out above, it is secreted by the same renal tubular mechanism which secretes paraaminohippurate, diodrast, phenol red, and penicillin. However, secretory transport may be dissociated from diuretic activity by the administration of probenecid, a drug which blocks tubular transport without affecting diuretic activity. Little or no chlorothiazide penetrates muscle, fat, brain or aqueous humor. However, it is secreted into the bile in appreciable amounts following oral administration. In contrast to acetazoleamide, chlorothiazide is not concentrated in erythrocytes, cellular concentrations in general are less than plasma concentration and drug is not retained in any tissue. Acetazoleamide, dichlorphenamide, and chlorothiazide all exert their major diuretic effects within the first 6 hrs. after oral administration.

***Alterations in Body Fluid Composition.*** Characteristically carbonic anhydrase inhibitors depress plasma pH; first, because they interfere with pulmonary excretion of carbon dioxide (in the dog more than in man, and acetazoleamide more than chlorothiazide); second, because they induce urinary excretion and reduce plasma concentration of bicarbonate. They likewise promote urinary loss of postassium, presumably by interfering with the supply of hydrogen ions to the exchange mechanism of distal tubular cells.

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net effect is a modest daily loss of sodium and chloride ions although the drug per se causes loss of sodium and bicarbonate ions.

A somewhat less desirable regimen is the administration of 0.25 to 0.5 gm. t.i.d. for a period of 2 to 3 days, whereupon the average patient becomes refractory. The drug is withheld for 2 to 3 days, and the course repeated. Correction of acidosis and excretion of chloride occurs in the drug free interval. Where production of hyperchloremic acidosis as a means of potentiating mercurial diuretics is the desired end, Luckey advises the daily administration of 0.75 gm. of acetazoleamide and up to 10 gm. of ammonium chloride in divided doses for 3 days; acetazoleamide is withdrawn but ammonium chloride is continued on the 4th and 5th days; and 2 ml. of Mercuhydrin are given on the 5th, 6th, and 7th days. This regimen has been particularly effective in diuretic resistant patients in congestive failure. Most agree that the administration of acetazoleamide and a mercurial diuretic on the same day reduces the response obtained with either agent. Luckey finds the peak diuretic response to mercury 48 hr. after the last dose of acetazoleamide. Where acetazoleamide is administered for its primary diuretic action, ammonium chloride is of course contraindicated, for it merely hastens the development of refractory acidosis.

It has been pointed out in Chapter XII on steroid therapy that the administration of ACTH, cortisone, hydrocortisone, prednisone, and prednisolone potentiate the diuretic actions of both organomercurial and sulfonamyl compounds. The mechanism of this potentiation is by no means clear. However, the administration of prednisone or prednisolone increases the response to acetazoleamide in the patient otherwise refractory to the drug. It seems likely that these steroids would also potentiate the action of dichlorphenamide and chlorothiazide.

*Dichlorphenamide* has been little studied clinically. Hence there is insufficient experience upon which to base dosage and therapeutic regimen. It is probable that this drug should be employed in much the same manner as acetazoleamide.

*Chlorothiazide* is ordinarily administered in a total daily dose of 0.5 gm. to 2.0 gm., in the lower range as a single dose, in the

anorexia or dietary fad, potassium may be drained from tissues to urine. Cells become depleted of potassium and gain sodium. Abdominal distension, weakness, lassitude, and cardiac irregularities develop. Whenever carbonic anhydrase inhibitors are used, it is wise to ensure an adequate potassium intake, e.g., 8 ounces or more of orange juice or 2 to 5 gm. of KCl per day.

All refractoriness to carbonic anhydrase inhibitors, does not derive from metabolic acidosis. Undoubtedly a significant factor is the reduction in glomerular filtration rate which occurs in response to a reduction in extracellular volume. Reduction in extracellular volume no doubt stimulates aldosterone secretion and enhances tubular reabsorption of sodium. Both responses increase glomerulo-tubular imbalance and favor retention of salt and water. The hypokalemia which develops in response to continued exhibition of a potent carbonic anhydrase inhibitor seems actually to stimulate the distal tubular mechanisms for acidification of the urine and for ammonia secretion, and hence contributes to the development of refractoriness.

*Dosage and Route of Administration.* Acetazoleamide, dichlorphenamide, and chlorothiazide are readily and rapidly absorbed on oral administration. While it is true that diuretic responses are more immediately impressive when a given dose is administered intravenously than when administered orally, one of the major virtues of these agents is lost if one resorts to parenteral therapy. If response to oral therapy is inadequate, it is better to turn to more potent agents rather than to intravenous administration.

*Acetazoleamide* may be administered in one of two ways to avoid, in whatever degree possible, the development of drug tolerance. A dose of 0.25 to 0.75 gm., preferably 0.5 gm., can be given once a day in the morning. The diuretic response lasts for 6 hr. or less, and during the remainder of the day disturbances in acid base balance are corrected by increased excretion of ammonia and titratable acid. Thus a response may be obtained each day under favorable circumstances, i.e., tolerance may not develop. The rationale of such treatment is the following: sodium and bicarbonate ions are excreted during the period of diuresis; chloride is excreted along with ammonia during the drug free interval. The

net effect is a modest daily loss of sodium and chloride ions although the drug per se causes loss of sodium and bicarbonate ions.

A somewhat less desirable regimen is the administration of 0.25 to 0.5 gm. t.i.d. for a period of 2 to 3 days, whereupon the average patient becomes refractory. The drug is withheld for 2 to 3 days, and the course repeated. Correction of acidosis and excretion of chloride occurs in the drug free interval. Where production of hyperchloremic acidosis as a means of potentiating mercurial diuretics is the desired end, Luckey advises the daily administration of 0.75 gm. of acetazoleamide and up to 10 gm. of ammonium chloride in divided doses for 3 days; acetazoleamide is withdrawn but ammonium chloride is continued on the 4th and 5th days; and 2 ml. of Mercuhydrin are given on the 5th, 6th, and 7th days. This regimen has been particularly effective in diuretic resistant patients in congestive failure. Most agree that the administration of acetazoleamide and a mercurial diuretic on the same day reduces the response obtained with either agent. Luckey finds the peak diuretic response to mercury 48 hr. after the last dose of acetazoleamide. Where acetazoleamide is administered for its primary diuretic action, ammonium chloride is of course contraindicated, for it merely hastens the development of refractory acidosis.

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higher, divided into 4 doses per day. As much as 8.0 gm. per day has been given for short periods with no adverse effects. However, no greater response is obtained with 8.0 gm. than with 2.0 gm. Tolerance does not develop to chlorothiazide in the same sense that it does to acetazoleamide, i.e., as a consequence of development of metabolic hyperchloremic acidosis. The reason is clear; chlorothiazide is a rather ineffective inhibitor of carbonic anhydrase and in the usual daily dose does not cause the excretion of appreciable quantities of bicarbonate. The clinical diuretic efficacy of chlorothiazide depends on its capacity to promote sodium and chloride excretion, not sodium and bicarbonate excretion. There is some tendency for chlorothiazide to cause the development of hypochloremic alkalosis of insignificant proportions. The alternation of courses of chlorothiazide and acetazoleamide or dichlorphenamide, the coadministration of the drugs or even the combination of chlorothiazide with small doses of ammonium chloride might be expected to correct this abnormality and render drug action more effective. Bayliss, Laragh and others recommend the coadministration of chlorothiazide and mercurial diuretics in diuretic resistant patients.

Chlorothiazide is of course as susceptible to nonacidotic mechanisms of drug refractoriness as any other diuretic. That is, low glomerular filtration rate, high rate of aldosterone secretion and hypokalemia all conspire to reduce diuretic activity.

*Toxicity.* The longer a drug is used, the broader becomes the spectrum of its toxic manifestations. Toxic actions of acetazoleamide are therefore recognized as more numerous than are those of chlorothiazide and dichlorphenamide, if for no other reason, because it has been used for a longer period of time.

If as much as 1.0 gm. of acetazoleamide is administered per day for any significant period of time, minor toxic manifestations are prone to develop, including anorexia, nausea, vomiting, diarrhea, paresthesias of face and extremities, lassitude, and drowsyness. Occasionally light sensitive skin rashes and rarely bone marrow depression results. Chlorothiazide, in comparison, has been described as occasionally producing gastric discomfort, mild paresthesias and skin rashes, but, in general, to be remarkably free of adverse

side reactions. Animal studies indicate that dichlorphenamide is a remarkably benign agent. However, it has not been sufficiently used in man to define its toxic manifestations when employed in diuretic therapy. All three drugs can produce potassium depletion and signs and symptoms of hypokalemia. Depletion of potassium is especially marked in patients with cirrhosis and evidence of marked liver dysfunction. Acetazoleamide and probably dichlorphenamide and chlorothiazide cause a significant increase in blood ammonia in patients with chronic liver disease. These two factors may precipitate hepatic coma in patients with impending precoma. Sherlock advises supplementation of dietary potassium in all patients with cirrhosis and ascites treated with acetazoleamide or chlorothiazide as a prophylaxis against potassium depletion and hepatic coma. Potassium supplements should also be given when chlorothiazide is used in the treatment of hypertension, for prolonged therapy with sulfonamyl compounds may cause potassium depletion in non-edematous as well as edematous patients. Digitalis toxicity is especially prone to occur in patients treated with sulfonamyl compounds. It is therefore wise to supplement potassium intake upon institution of diuretic therapy. Hyponatremia may develop as a consequence of salt restriction and excessive fluid intake and is managed as described in Chapter XIX. Daily doses of acetazoleamide may produce a marked metabolic acidosis in patients with severe renal insufficiency, a complication less apt to occur with chlorothiazide because it is less effective as an inhibitor of carbonic anhydrase. Dichlorphenamide will probably prove similar to acetazoleamide in this respect.

*Comparison of Potentialities of Acetazoleamide, Dichlorphenamide, and Chlorothiazide.* Acetazoleamide, dichlorphenamide, and chlorothiazide are useful drugs when properly employed in selected patients. Experience with dichlorphenamide and chlorothiazide has been insufficient to justify a truly valid comparison, although limited clinical experience indicates that the latter drug is considerably more potent and more widely effective than acetazoleamide. Acetazoleamide finds its greatest use in states of moderate salt retention, not in severely ill patients maximally retaining sodium. The nature of the disease process seems much

less significant than its severity. The need for a potent diuretic is, of course, related to intensity of salt retention, thus to severity of the disease process. However, the usefulness of an oral diuretic agent is related to its freedom from undesirable side reactions, its ability to control moderate salt retention, thus to liberalize the dietary regimen, and its capacity from day to day to maintain a stable edema free state. In certain patients, acetazoleamide accomplishes these ends admirably. General experience has been that the severely ill patient responds inadequately.

Chlorothiazide gives promise of greater efficacy in patients with more intense salt retention. Ford, Laragh, Schreiner, Bayliss and others find it effective in a variety of edematous states and in some patients refractory to other forms of diuretic therapy. Whether it will supplant mercurial diuretics, potentiated with ammonium chloride, aminophylline, and/or corticosteroids in the treatment of severely ill patients must be decided on the basis of wider clinical trial.

Certain physiological considerations incline one to favor chlorothiazide over acetazoleamide or dichlorphenamide as a diuretic apart from the ultimate criterion of clinical efficacy. A diuretic which blocks reabsorption of chloride and sodium ions should be more effective in the treatment of edema than one which blocks reabsorption of bicarbonate and sodium ions. The reasons for this are simple. (1) The filtered load of chloride is some four times the filtered load of bicarbonate. Were one to block 10 per cent of the reabsorption of chloride and sodium, four times as much edema fluid would be removed as if one were to block 10 per cent of bicarbonate reabsorption. (2) If instead one considers the effect of the urinary loss of 140 mEq. of sodium as bicarbonate, the distortion of the acid-base patern of the extracellular fluid would be greater than if the same amount of sodium were lost as chloride. (3) The distortion of the acid-base pattern produced by loss of sodium and chloride (metabolic alkalosis) can be easily corrected by administration of ammonium chloride, which in effect adds neither anion nor cation to the body fluids, but merely substitutes chloride for bicarbonate. The distortion produced by loss of sodium and bicarbonate can be corrected only by giving sodium

bicarbonate or some sodium salt with a metabolizable anion (neither procedure is desirable) or by relying on the kidneys to correct the imbalance, which they may do with little or no net loss of fixed cation.

## SUMMARY

N' unsubstituted sulfonamyl compounds inhibit carbonic anhydrase and thus depress the hydration of carbon dioxide to form carbonic acid. It is probable that these drugs reduce the availability of hydrogen ions to both proximal and distal tubular ion exchange mechanisms. The proximal tubular mechanism exchanges hydrogen ions for sodium ions in the process of reabsorbing sodium bicarbonate. Enzymatic blockade of this system results in the excretion of sodium and bicarbonate ions. The distal mechanism exchanges either hydrogen or potassium ions for sodium ions in processes concerned with reabsorption of bicarbonate, with acidification of the urine, and with secretion of potassium and ammonia. Enzymatic blockade of this system results in alkalinization of the urine, in the excretion of additional sodium and bicarbonate ions, and in the secretion of potassium rather than hydrogen ions or ammonia.

Inhibition of carbonic anhydrase depends on the presence within the drug molecule of an unsubstituted sulfonamyl group, $-SO_2 \cdot NH_2$. Heterocyclic compounds, such as acetazoleamide, or halogen substituted disulfonamides, such as dichlorphamide, are most active. Sulfonamyl benzothiadiazines, such as chlorothiazide and perhaps dichlorphenamide as well possess an additional property, namely, the ability to block the proximal tubular reabsorption of sodium and chloride ions. The clinical efficacy of chlorothiazide as a diuretic depends on this latter property, its capacity to inhibit carbonic anhydrase is weak and it rarely causes excretion of bicarbonate or alkalinization of the urine when given orally to patients.

Acetazoleamide, by blocking the reabsorption of sodium bicarbonate, produces metabolic hyperchloremic acidosis. Acidosis is a major cause of the refractoriness which develops when acetazoleamide is administered in repeated doses. Chlorothiazide, by blocking the reabsorption of sodium chloride produces metabolic hypo-

chloremic alkalosis. This disturbance of acid base balance is relatively mild and does not seem to alter drug efficacy. It is readily correctible by coadministration of acetazoleamide, dichlorphenamide, or small doses of ammonium chloride. Dichlorphenamide is claimed on the basis of animal experiments to induce drug refractoriness to a lesser extent than acetazoleamide. Whether this will be borne out in clinical usage is unknown. Reduction of filtration rate and excessive secretion of aldosterone contribute to refractoriness to all sulfonamyl compounds. Mercurial diuretics may be administered in association with chlorothiazide but not with acetazoleamide. Acetazoleamide, chlorothiazide and dichlorphenamide are relatively benign and when given in proper dosage produce few side reactions. However, *all sulfonamyl compounds may cause depletion of body stores of potassium, and it is imperative that dietary intake be adequate when these drugs are administered over prolonged periods of time.*

Acetazoleamide produces adequate diuresis in mild salt retaining states but is frequently ineffective in more severely ill patients. Limited clinical experience indicates that chlorothiazide may produce diuresis in more severely ill patients. The severity of the disease process rather than its nature appears to be the limiting factor in the therapy of edema with any of these agents. Clinical usage of dichlorphenamide has not been sufficient to assess its potentialities.

## REFERENCES

1. Berliner, R. W.· Carbonic anhydrase inhibitors. *Pharmacol. Rev*, *8*:137, 1956.
2. Gilman, A.. The role of the kidney in the regulation of acid base metabolism. *Am. J. Med.*, *15*:765, 1953.
3. Kessler, R. H., Hierholzer, K., Gurd, R. S., and Pitts, R. F.· Localization of the actions of chlorothiazide in the nephron of the dog. *Am. J. Physiol.*, *196*:1346, 1959.
4. Pitts, R. F.: Renal excretion of acid. *Federation Proc.*, 7:418, 1948.
5. Pitts, R. F.: Acid-base regulation by the kidneys. *Am. J. Med.*, *9*:356, 1950.
6. Pitts, R F.: Mechanisms for stabilizing the alkaline reserves of the body. *Harvey Lect.*, *48*:172, 1952-53.

7. PITTS, R. F.: Some reflections on mechanisms of action of diuretics. *Am. J. Med.*, *24*:745, 1958.
8. PITTS, R. F., GURD, R. S., KESSLER, R. H., and HIERHOLZER, K.: Localization of acidification of urine, potassium and ammonia secretion, and phosphate reabsorption in the nephron of the dog. *Am. J. Physiol.*, *194*:125, 1958.
9. PITTS, R. F., KRÜCK, F., LOZANO, R., TAYLOR, D. W., HEIDENREICH, O. P. A., and KESSLER, R. H.: Studies on the mechanism of diuretic action of chlorothiazide. *J. Pharmacol & Exper. Therap.*, *123*:89, 1958.
10. Symposium on Chlorothiazide and other diuretic agents. *Ann. New York Acad. Sc.*, *71*:32, 1958.

*Chapter XVIII*

# POTASSIUM SALTS

POTASSIUM salts are among the oldest of diuretics. Thom Willis in 1679 first recommended the administration of potassi nitrate in dropsy, and even today the nitrate is accepted as t most effective salt. The order of efficacy is claimed to be nitra chloride and bicarbonate, acetate, and citrate, the last three havi equal diuretic potency. All of these salts may produce loss sodium and water and decrease in body weight when administe in adequate dosage to edematous patients. Because many patie respond in an inadequate fashion, potassium salts are rarely u today as primary diuretics. However, they are valuable adjuva in therapeutic regimens which include more potent diuretics, for such drugs increase urinary excretion of potassium. If diet intake is inadequate to cover urinary losses, hypokalemia a depletion of cellular stores of potassium occur. Muscular weakn cardiac irregularities, anorexia, and abdominal distension, associa with diuretic therapy, are frequent results of depletion of po sium stores. Digitalis intoxication in the cardiac optimally digi ized prior to diuretic therapy and precipitation of hepatic co in the cirrhotic can likewise be assigned in many instances potassium depletion. Potassium supplementation is especially nec sary when daily doses of acetazoleamide, chlorothiazide, or dichl phenamide are administered.

*Mechanism of Diuretic Action.* According to Berliner, most the potassium entering the renal tubule in the glomerular filtr is reabsorbed in the proximal segment; that which is excreted the urine is in large part added in the distal portion of the nephr It is probable that some potassium is secreted in the collect duct. As pointed out in Chapter IV, potassium ions are secre into the urine in exchange for sodium ions by the same di

mechanism which secretes hydrogen ions. When potassium chloride is administered in adequate dosage, the urine becomes alkaline and contains increased amounts of potassum, bicarbonate and sodium. Chloride excretion is only slightly increased. If potassium nitrate or sulfate are administered, somewhat more chloride is eliminated.

These findings are most adequately explained by the now generaly accepted thesis that hydrogen and potassium ions compete for a common distal transport mechanism. If large numbers of potassium ions are supplied to the exchange mechanism, potassium rather than hydrogen is transported, and the urine becomes alkaline. According to Berliner, Kennedy and Orloff, competition between potassium and hydrogen ions for the common exchange mechanism is not on a one to one basis; instead it is roughly on a two to three basis. Thus for the secretion of every two potassium ions, three hydrogen ions are displaced from the exchange mechanism. One less sodium ion and three fewer bicarbonate ions are reabsorbed. There results a net loss of one sodium ion from body stores for each two potassium ions secreted. The excess cations are largely eliminated as bicarbonate. Because hydrogen ion exchange is depressed and the urine becomes alkaline, ammonia excretion declines. Continued oral administration of potassium salts therefore results in the development of a mild hyperchloremic metabolic acidosis. The acidosis is self-limited, for in the course of a few days chloride replaces bicarbonate in the urine. The diuretic action of potassium salts is also evanescent; within a few days sodium loss in the urine ceases.

Potasium salts also act as osmotic diuretics (*cf.* Chapter XIV). Such efficacy as they may have in this respect is due to their rapid elimination by tubular secretion. In the postabsorptive state, the potassium clearance of the normal individual is less than one-quarter the rate of glomerular filtration, a fact which indicates extensive tubular reabsorption. Following ingestion of a potassium salt, clearance rises sharply with relatively little increase in serum concentration to approach and even to exceed glomerular filtration.

*Dosage.* Potassium salts have been administered orally in daily doses of 5 to 10 gm. of the chloride; 8 to 12 gm. of the nitrate,

and up to 20 gm. of the citrate. They may be given in divided doses 3 to 4 times daily in 10 per cent solution or in capsules. In general, the greater the dosage, the more adequate the response, but at best results are not impressive. Within the dose range noted above, potassium salts are well tolerated by most patients, and cause only minimal changes in serum concentration. This latter finding results from the fact that potassium penetrates cells readily, hence distributes throughout total body water; intracellular concentration increases in proportion to the increase in extracellular concentration. However, in some patients, potassium salts cause gastric irritation. Because of their brief diuretic action, potassium salts are commonly administered for 3 to 4 days, followed by a rest period of equal length. When given prophylactically, they are administered daily.

*Contraindications.* Potassium salts should not be given to patients with markedly impaired renal function and elevated serum potassium levels, because of the danger of potassium intoxication with its manifestations of cardiac conduction disturbances and cardiac arrest. Renal disease per se is not a contraindication to use of potassium salts for the capacity to excrete potassium is well maintained until late in the disease process.

## SUMMARY

Potassium salts are weak diuretics by virtue of the fact that the exchange of hydrogen ions for sodium ions in the distal portion of the nephron is depressed to a greater degree than the exchange of potassium ions for sodium ions is enhanced. They also act as mild osmotic diuretics. The major use of potassium salts in diuretic therapy is in prophylaxis of hypokalemia and depletion of cellular potassium reserves.

## REFERENCES

1. Berliner, R. W., Kennedy, T. J. and Orloff, J.: Relationship between acidification of the urine and potassium metabolism *Am. J. Med.*, *11*:274-282, 1951.
2. Davidson, D. G., Levinsky, N. G., and Berliner, R. W.: Maintenance of potassium excretion despite reduction of glomerular filtration during sodium diuresis. *J. Clin. Invest.*, *37*:548, 1958.

3. Pitts, R. F. and Sartorius, O. W.: Mechanism of action and therapeutic use of diuretics. *J. Pharmacol. & Exper. Therap.* II, *98*:161-226, 1950.

## *Chapter XIX*

# HYPONATREMIA AND POTASSIUM DEFICIENCY

REDUCTION of the sodium concentration of extracellular fluid (hyponatremia) and depletion of body reserves of potassium (associated with hypokalemia) are complications which may arise in the course of therapy with any effective diuretic agent. These alterations in composition of the body fluids increase disability, interfere with reduction of edema, and when severe, jeopardize life. Within limits, they can and should be avoided; if they occur, they should be recognized and treated appropriately.

### HYPONATREMIA

Hyponatremia in clinical parlance means a reduction in the plasma or serum concentration of sodium to a value less than 135 mEq. per liter, the lower limit of normal, although the term literally means a reduction in the sodium concentration of blood. Actually hyponatremia is a clinically significant cause of symptoms and signs (low salt syndrome) only when concentration falls below 125 mEq. per liter. Furthermore, as Albrink et al have pointed out, some degree of hyponatremia is entirely normal in marked hyperlipemia. Lipid occupies volume in plasma, sodium is dissolved in water. Accordingly, the sodium concentration in the aqueous phase of plasma, hence in extracellular fluid, may be normal, yet concentration per liter of plasma may be subnormal in proportion to volume occupied by lipid. Even in true hyponatremia, symptoms and signs referable to this abnormality are conditioned both by the rate at which it develops and by its inciting cause. We shall confine this discussion to hyponatremia associated with edema and/or ascites which develops in the course of and often as a consequence of therapy. Welt distinguishes three types of hypona-

tremia, chronic hyponatremia, acute hyponatremia without evidence of reduction in plasma volume, and acute hyponatrema with evidence of peripheral circulatory failure. All are fundamentally dilutional in origin, i.e., water is retained in the body in excess of sodium, or conversely sodium is excreted in excess of water.

*Chronic Hyponatremia* develops gradually and is most frequently observed in nephrosis, cirrhosis with ascites, and in severe and long standing congestive circulatory failure. It also occurs late in the course of many severe debilitating diseases unassociated with edema, e.g., advanced pulmonary tuberculosis and generalized carcinomatosis. It may be an expression of wide spread depression of metabolic functions of all cells. Since cachexia is often a common factor, it has been loosely termed the "sick cell" syndrome. Chronic hyponatremia per se is singularly asymptomatic. Little evidence of cellular edema exists and attempts to correct the obvious hypoosmolality of extracellular fluid by the infusion of hypertonic sodium chloride causes intense thirst; when water is ingested, body fluids are rediluted. Those patients unable to excrete a salt load, i.e., those with edema or ascites, expand extracellular and transcellular fluid volumes. Those patients capable of excreting a salt load do so and return to their pretreatment condition. In any event chronic hyponatremia is a grave sign.

The cause of chronic hyponatremia is not known with any certainty. However, one may speculate that it represents a primary reduction in osmolality of cells and an associated secondary reduction in osmolality of extracellular fluid. Presumably the osmotic activity of the contents of the intracranial osmoreceptors, like that of other cells, is reduced. Therefore, they respond to concentration and dilution of extracellular fluid in an abnormal fashion; i.e., the osmostat (analogous to a thermostat) is reset to a lower level.

Laragh has shown that the oral administration of 4 to 22 gm. of KCl to chronically hyponatremic edematous patients may cause a striking increase in the extracellular sodium concentration without the administration of exogenous sodium and without loss of water. Potassium enters cells in exchange for sodium. If more potassium enters than sodium leaves, cellular osmolality increases, and water

enters by osmosis. The transfer of sodium and possibly hydrogen ions to extracellular fluid in exchange for potassium and the migration of extracellular water into cells both conspire to increase extracellular osmolality and to raise plasma sodium concentration How universally potassium therapy will correct hyponatremia is not known. The procedure is not without hazard for it appears that the chronically edematous hyponatremic patient is less capable of excreting an excessively large potassium load than is the non-hyponatremic patient. Elevation of plasma potassium is therefore prone to occur.

Chronic hyponatremia is commonly associated with resistance to diuretic therapy, the usual regimens causing neither loss of water nor loss of sodium. As was pointed out in Chapter XII, Schemn first observed that certain edematous, hyponatremic, and moribund patients, resistant to all common forms of therapy, could be made responsive by treatment with ACTH and cortisone. Subsequently others showed that prednisone and prednisolone were also effective in potentiating diuretic activity. Often steroids first induce water diuresis, restoring osmolality toward normal, then in combination with organomercurial or sulfonamyl compounds induce sodium and water diuresis, causing further reduction of edema. It is not known if the primary action of steroids is to promote the excretion of water by a direct renal action, resulting secondarily in an increase in extracellular and cellular osmolality, or if their primary action is to increase cellular osmolality, including a resetting of the osmostat, resulting secondarily in a loss of water from the extracellular compartment.

As has been pointed out in preceding chapters, various procedures may be employed to increase diuretic efficacy in resistant patients, including those with chronic hyponatremia. However, addition of salt to the diet or the infusion of hypertonic saline is relatively ineffective either in correcting hyponatremia or in restoring responsiveness to diuretics.

*Acute Dilutional Hyponatremia Without Reduction in Plasma Volume* commonly develops at an intermediate rate in patients maintained on a low salt diet, subjected to intensive diuretic therapy, and encouraged to drink excessive amounts of water.

Except for the presence of edema, the condition is akin to that which develops in normal subjects maintained on a low salt intake, depleted of sodium by profuse sweating, and allowed free access to water, as described by McCance and others. Hemoconcentration and signs of peripheral circulatory failure are absent, but the patient is restless, complains of muscle cramps, exhibits muscle twitching, becomes lethargic or comatose, and finally convulses. Symptoms and signs are those of water intoxication and in large part may be assigned to cellular edema. Superimposed on this picture may be evidence of progressive renal failure, with reduced glomerular filtration rate and elevated blood urea nitrogen.

It was pointed out in Chapter V that osmolality and volume of extracellular fluid are regulated by semi-independent mechanisms. However, it was further stated that interaction occurs and that precise regulation of osmolality may be sacrificed in the interest of partial maintenance of volume. The patient with an absolute or relative reduction in plasma or extracellular fluid volume exhibits thirst, drinks water and partially restores volume at the expense of reduced extracellular and cellular osmolality. Two possible causes of water retention have been suggested. First, the volume receptor mechanism acting through the osmoregulatory-antidiuretic hormone mechanism, stimulates retention of water, even though osmolality of the body fluids is depressed. Second, the volume receptor mechanism, acting through autonomic or humoral effector mechanisms, reduces glomerular filtration rate. As Berliner and others have shown, a reduction of filtration rate of sufficient magnitude will cause over-reabsorption of water and formation of small volumes of hypertonic urine, even though the body fluids are excessively diluted. It is not known which of the two mechanisms is the more significant; both might well play a role.

If the symptoms and signs of acute dilutional hyponatremia are mild, withholding of water, cessation of diuretic therapy, and liberalization of salt intake may be all that is required. If moderate, hypertonic urea may be given by gavage to abstract water from the body osmotically. If severe, hypertonic saline, given intra-

venously, is necessary and often provides immediate and dramatic relief.

*Acute Dilutional Hyponatremia With Evidence of Peripheral Circulatory Failure* develops shortly after massive paracentesis, profound diuresis, and severe vomiting or diarrhea. The clinical picture resembles that described above but includes in addition, symptoms and signs of peripheral circulatory collapse. Fall in blood pressure, rapid, weak, thready pulse, cold, clammy cyanotic skin, oliguria or anuria, and hemoconcentration are added to the signs of water intoxication.

The removal of large volumes of ascitic fluid reduces intra-abdominal pressure. Transudation occurs at an accelerated rate and plasma volume is reduced. Thirst drives the patient to replace his fluid deficit, but most of the water ingested enters cells, interstitial fluid, and the peritoneal cavity. Body fluids are diluted, only a small fraction of the water is retained in the vascular bed, and peripheral circulatory failure results. Massive diuresis, vomiting, or diarrhea may similarly result in reduced circulating blood volume, thirst and dilution of body fluids. The factors which limit the excretion of water and restoration of osmolality of body fluids are those described above. Treatment is that for acute dilutional hyponatremia without evidence of reduction in plasma volume.

The occurence of acute dilutional hyponatremia with or without evidence of peripheral circulatory collapse can frequently be avoided by repeated paracentesis of small volumes of fluid (2 or 3 liters) rather than by a single massive abdominal tap. Similarly, diuretics should be administered to massively edematous patients in such amounts as to remove not more than 2 to 3 liters of edema fluid per day. The more profound the diuresis, the greater is the incidence of complications.

*Hypertonic Saline in the Treatment of Hyponatremia* in edematous patients should be employed with caution and only in the presence of symptoms and signs of frank water intoxication: lethargy, coma, muscle twitching, or convulsions. In less critical situations, the withholding of fluids, cessation of diuretic therapy, and liberalization of salt intake will ameliorate the condition.

According to Welt the quantity of sodium required to restore

osmolality of the body fluids to normal is equal to the deficit in concentration per liter of plasma multiplied by the estimated number of liters of total body water. This does not imply, as one might surmize, that sodium is distributed throughout body water, cellular as well as extracellular. Rather it implies a uniform osmolality throughout all body fluids which demands that an increase in the concentration of sodium in extracellular fluid be accomplished by the same increase in total solute concentration in the cell water. This increase in intracellular concentration will be accomplished by the movement of water from the cells in response to the addition of hypertonic salt to the extracellular fluid. Although the administered salt will, for the most part, be confined to the extracellular compartment, its osmotic effect is distributed throughout both compartments. In terms of mEq., it is advisable to administer one-fifth of the dose as sodium bicarbonate, four-fifths as sodium chloride to avoid acidosis. It must be given intravenously at a slow rate in hypertonic solution, 3 to 5 per cent, and water must be withheld for the succeeding 6 to 12 hr. to prevent redilution and increased edema. It is preferable to give only a part of the calculated dose and to note the response. Marked clinical improvement will occur with partial correction of hyponatremia if symptoms and signs are in truth due to this abnormality of body fluid composition.

## HYPOKALEMIA AND POTASSIUM DEFICIENCY

Depletion of body stores of potassium is also a potential complication of all forms of diuretic therapy. It is most prone to develop in the course of prolonged or intensive treatment with mercurial diuretics, hydrogen and ammonium cycle resins, ammonium chloride, and sulfonamyl compounds, especially if anorexia or vomiting limit dietary intake or if diarrhea causes increased fecal loss. Under most circumstances, hypokalemia, i.e., reduction of the plasma concentration of potassium to a value less than 3.5 mEq. per liter, is indicative of potassium depletion. However, the extent of the reduction in plasma potassium is no adequate gauge of the magnitude of total body deficit.

*Manifestations of Potassium Depletion* include the neuromuscular signs of diminished deep reflexes, weakness progressing to flaccid paralysis, and mental confusion; the gastrointestinal signs of anorexia, diarrhea, abdominal distension, and ultimately paralytic ileus; the cardiac signs of rapid rate and irregular rhythm; and the renal signs of isosthenuria, and less frequently, reduction in filtration rate and azotemia.

Potassium depleted patients are highly sensitive to the digitalis glycosides. Those who are on maintenance doses and who are adequately digitalized show signs of toxicity if potassium stores are even modestly depleted. Electrocardiographic abnormalities of potassium depletion and/or digitalis intoxication include low or inverted and broadened T waves, prolongation of the Q-T interval, and sagging and finally depression of the S-T segment. Increased excitability and abnormalities of impulse initiation and conduction may become evident Potassium ions antagonize the effects of digitalis glycosides on the heart; calcuim ions potentiate them. Therefore either potassium depletion or calcium excess will induce digitalis toxicity.

Isosthenuria, polyuria, and insensitivity to ADH are the major functional manifestations of renal potassium depletion and are more or less reversible with therapy. Potassium depleted kidneys seem especially vulnerable to the development of pyelonephritis Reduced glomerular filtration rate, azotemia and even acute renal failure have been described as resulting from potassium depletion and as responding favorably to potassium repletion.

In animals experimentally depleted of potassium, lesions have been described in skeletal muscle, cardiac muscle, and the Purkinje system. Muscle fibers exhibit edema and fragment, focal areas of necrosis develop, and the degenerated regions become fibrotic. Fibers of the Purkinje system show granulation and vacuolation. Somewhat similar lesions have been observed in patients who succumb in diabetic ketosis, diarrheal diseases, and other conditions associated with potassium depletion Renal lesions are confined mainly to the tubular epithelium and include swelling, hyperplasia, granulation, and vacuolation. Changes are most marked and appear earlier in the collecting ducts. In the experimental

animal, swelling and hyperplasia of clear cells and proliferation of intercalated cells may obstruct collecting ducts and lead to dilation of more proximal portions of the nephron. Cytological changes are observed in the proximal and distal tubules in long standing potassium depletion. Despite the functional derangements of the gastrointestinal tract and central nervous system, no striking pathological changes occur in gut, brain or spinal cord.

In severe hepatic insufficiency the capacity of the liver to remove ammonium ion from the portal blood is reduced and accordingly the concentration of this ion in systemic blood increases. In hypokalemia the partial pressure of free ammonia ($PNH_3$) rises due to the associated alkalosis. Apparently toxicity is more related to $PNH_3$ than to total ammonia plus ammonium ion concentration, for cells are far more permeable to free ammonia than to the ion. Potassium deficiency is a predisposing cause of hepatic coma in liver disease and its deleterious effect is in part due to alkalosis and increased $PNH_3$.

*Maintenance of Normal Intracellular Potassium Ion Concentrations* depends on the continuous active transport of potassium into cells and the continuous active extrusion of sodium from cells, processes discussed in some detail in Chapter II. If potassium is lost from extracellular fluid into urine, vomitus, or feces, the normal steady state relationship is disturbed As Darrow and others have shown, potassium ions move out of cells to replace extracellular losses and sodium and hydrogen ions enter cells These ion shifts result in extracellular hypokalemic alkalosis and intracellular acidosis If on the other hand an acid load is imposed on the body, the acid is buffered in part by the entry of hydrogen ions into cells in exchange for potassium ions. The potassium lost by cells is excreted in the urine.

*Depletion of Cellular Stores of Potassium in the Course of Diuretic Therapy* may be explained in terms of one of the other of the two mechanisms outlined above. Ammonium or hydrogen cycle resins have a greater affinity for potassium than for sodium. As pointed out in Chapter X, they bind significant quantities of potassium in the gut. Furthermore, the colonic mucosa of edematous patients actively conserving sodium is stimulated by the high

titre of circulating aldosterone to exchange potassium derived from extracellular fluid for fecal sodium bound to resin. Potassium lost in the feces is replaced from cellular stores. To prevent progressive depletion of cellular potassium, at least one-third of the dose of resin is commonly administered in the potassium cycle.

Ammonium chloride is converted in the liver to urea and hydrochloric acid. As pointed out in Chapter XI, an appreciable fraction of this acid is neutralized by intracellular buffers, hydrogen ions entering cells in exchange for potassium ions. The potassium is excreted in the urine to maintain extracellular concentration within the narrow limits of normal. Accordingly, cellular stores are reduced, and if dietary intake is inadequate, serious depletion may result.

Mercurial diuretics inhibit a limited fraction of the proximal tubular reabsorption of sodium and chloride ions. In edematous patients, circulating aldosterone stimulates the ion exchange mechanisms located in the distal tubule and collecting ducts. The excess sodium which enters the terminal part of the nephron from the proximal segment may be exchanged in large part for potassium, hydrogen and ammonium ions. The chloride is excreted in association with these ions rather than sodium. Osmotic diuretics, which interfere with proximal reabsorption of sodium and chloride ions, may likewise induce the excretion of potassium because of enhanced distal exchange.

The sulfonamyl inhibitors of carbonic anhydrase more directly promote potassium loss by reducing the availability of hydrogen ions to the exchange mechanism. Blockade of exchange of hydrogen and ammonium ions for sodium ions is compensated by increased exchange of potassium ions. The more intense the stimulation of the exchange mechanism by circulating aldosterone, the greater is the depletion of body stores of potassium.

***Treatment and Prophylaxis of Potassium Deficiency.*** The extent of depletion of postassium stores cannot be estimated in any way applicable to the routine management of patients. It can be measured in the course of balance studies by determining the quantity of potassium retained when daily oral supplements are administered over a prolonged period of time. It may also be

estimated by isotope dilution methods. There is, however, no need for exact knowledge of deficits; if any of the symptoms or signs of potassium depletion appear, supplementation of intake with 250 ml. or more per day of orange juice or 3 to 5 gm. per day of KCl will induce a remission of symptoms well before the deficit is corrected. The correction of large deficits, of the order of 400 to 1000 mEq. must be done slowly over a period of weeks. Prevention of potassium depletion by insuring adequate dietary intake is far preferable to treating deficiency when it occurs. Although potassium salts per se are not especially potent diuretics, their routine use along with more effective agents does no harm and guards against the development of deficits. Whenever sulfonamyl diuretics are used, potassium supplements should be given. This is equally true in the use of these agents in hypertensive therapy for sizable doses are administered daily over prolonged periods of time. Only in severe renal disease with basally elevated plasma potassium levels should potassium salts not be used. Under such conditions, there is usually no cause for diuretic therapy of any sort.

## SUMMARY

Hyponatremia and depletion of body stores of potassium may occur in the course of therapy with any effective diuretic agent. These alterations in ionic composition of the body increase disability, render treatment ineffective, and when severe, jeopardize life.

Acute hyponatremia may develop rapidly in response to massive paracentesis or profound diuresis or at an intermediate rate in the course of intensive diuretic therapy. It results from stimulation of antidiuretic mechanisms by reduction in circulating blood volume, and may be associated with signs of peripheral circulatory collapse. Thirst, ingestion of water, and inhibition of water diuresis cause dilution of body fluids. The condition may be avoided by less intensive diuretic therapy and by repeated abdominal taps of small volume rather than a single massive tap. Mild to moderate hyponatremia can be adequately managed by withholding water, stopping diuretic treatment, and liberalizing salt intake. Only if signs of water intoxication are severe should hypertonic saline be

therefore, increase the permeability of the connective tissue. This would be the effect also of those diffusion factors, which — generally *known under the name of* hyaluronidase — *belong* more or less purified testicular extracts and bacterial filtrates.

So long as the capillary filtrate has to diffuse through the ground substance in order to reach the parenchyma cells and the lymphatics, it is obvious that changes in the permeability of the basic structure of the connective tissue must very strongly *influence* the diffusion of water and possibly still more that of colloidal and corpuscular elements.

It had long been conjectured that processes of diffusion in the connective tissue did not occur in the same manner as in inanimate systems, however intricate their architecture. Not until Duran-Reynals (*1928*) had discovered the diffusion factor [subsequently identified as hyaluronidase by Chain and Duthie (1940)], did these conjectures assume a concrete form. It was then demonstrated by a number of authors that a variety of substances (e.g. testicular extract, bacterial filtrates, bee and snake poison, leech extract etc.) contained hyaluronidase and increased the permeability of the connective *tissue (e.g.* McClean 1936; Duran-Reynals 1942; etc.) Enzymes contained in these extracts act upon the ground substance of connective tissues and depolymerize the mucopolysaccharides there (hyaluronic acid, chondroitin sulfate, etc.). Such disaggregation induces the decomposition of hyaluronic acid which closes the pores of the basic structure of connective tissue; in other words, it changes from gel to sol with the result that connective tissue becomes more permeable not only to water and dissolved substances (crystalloids, colloids) but corpuscular particles (India ink, bacteria, tumour cells, etc.) as well.

Although the existence of the above-outlined mechanism has not *yet been proved with absolute certainly and in spite of the fact that* several data have been reported which do not agree with the theory, we are inclined to assume that hyaluronidase, or rather some other enzyme of similar effect, surely plays a role in regulating the physiological permeability of the connective tissue. The presence of the enzyme in various areas of the human and animal organism is demonstrable: *it is encountered not solely in the testicular extract but also in the* ciliary body, in the aqueous humour, the spleen etc. Claude and Duran-Reynals (1943), Meyer et al. (1941) showed that enzymes — in an inactive state — are present also in the subcutaneous connective tissue. We feel that we must emphasize that notwithstanding these reports, it cannot be regarded as a fact beyond any doubt that skin and subcutaneous connective tissue contain hyaluronidase. Commenting on the results of all those earlier authors who believed that they had proved the presence of hyaluronidase in the skin (e.g. Claude and Durán-Reynals 1934; Meyer et al. 1941; etc.), Glick and Grais (1948) raised the justified objection that they had only succeded *in isolating the diffusion factor from autolyzed* and long-incubated

skin, while it is not only possible but probable that the hyaluronidase was produced by bacteria during incubation.

Hyaluronidase, by the way, is not the sole agent which affects the permeability of connective tissues. It is presumably in the first phase only that interaction between hyaluronidase and hyaluronic acid plays a role in the spreading reaction, while other factors are at play in the subsequent phase (Duran-Reynals 1954). Of these let us mention the amount, viscosity and osmotic pressure of the fluid injected for the study of the spreading reaction; further the decomposition products of the hyaluronic acid itself which are also enhancing tissue permeability; increased permeability of the capillaries; etc. (Hechter 1950; Duran-Reynals 1952; Seifter and Baeder 1954; etc.).

## INFLUENCE OF SUBSTANCES WITH ANTI-HYALURONIDASE ACTION ON DIFFUSION IN THE CONNECTIVE TISSUE AND ON THE FORMATION OF OEDEMAS

In connection with the regulation of the hyaluronic acid—hyaluronidase system we attached a certain importance to those anti-invasive agents which are present in blood plasma even under normal [illegible]

in normal conditions, but certain diseases may considerably change it. For instance, the titre increases in cases of rheumatic fever (Friou and Wenner 1947; Quinn 1948; Harris et al. 1949; etc.), poliomyelitis (Glick and Gollan 1948), pneumonia (Thompson 1948), glomerulonephritis (Harris et al. 1950), shock (Cole, Shaw and Fraser 1950) and also in other diseases.

Increased anti-hyaluronidase titre *of the serum without a concomitant* increase in mucoprotein concentration has been found also in children suffering from lipoid nephrosis (Glick et al. 1949). This finding induced us to examine the anti-hyaluronidase titre in the serum of oedematous patients, for it was conceivable that a certain correlation existed between the appearance of oedema and the anti-hyaluronidase action of the serum. The first result of these investigations (Földi, Rusznyák, Szabó and Vágó 1951) was that each ml of normal human serum inhibits the action of about 18 to 20 viscosity-reducing units of hyaluronidase (approx. 0·5 mg Hyalase—Richter)

Using this method we found the serum of patients suffering from oedema of cardiac origin to have a perfectly normal anti-hyaluronidase titre, a finding confirmed by the fact that the serum of such patients contained almost exactly the same amount of hyaluronidase-inhibitor as the simultaneously analyzed blood of healthy individuals (Table 28).

TABLE 28

*Anti-hyaluronidase titre of serum in cardiac oedema*

| N° | AHU/ml | |
|---|---|---|
| | Card oed | Normal contr |
| 1 | 19 | 19 |
| 2 | 19 | 19 |
| 3 | 17·5 | 17·5 |
| 4 | 16 | 15 |
| 5 | 21 | 20 |
| 6 | 18 5 | 20 |
| 7 | 15 | 20 |
| 8 | 22 5 | 22 |
| 9 | 22 5 | 22 |

This is not so in cases of renal oedema. Table 29 makes it clear that, in agreement with the literature, in cases of genuine nephrosis, the concentration of the hyaluronidase-inhibitor is in fact fairly high, while anti-hyaluronidase titre is exceedingly low in nephritis and in pseudonephrosis which, from a clinical point of view, is very similar to genuine nephrosis. It was reasonable to suppose that the anti-hyaluronidase concentration of the serum may be influenced in these cases by disturbed renal function through either an accumulation of waste products in the blood or some other mechanism. In Table 29 we give, therefore, all other data concerning the analyzed cases. It shows that there exists no connection between the impairment of renal function and the anti-hyaluronidase titre of the serum, since the reduction of titre is associated with normal and pathological values of glomerular filtration and serum non-protein nitrogen alike. Nor does the change in anti-hyaluronidase titre depend on the serum's protein level:

n-

i-

bition was increased in one case and diminished in the other. The uniformly directed change of the protein composition excludes also the assumption that the lack of some protein fraction in the serum may

have led in one case to a reduced titre of the unspecific hyaluronidase inhibitor. It was in any case very striking to have found a very low anti-hyaluronidase concentration in patients suffering from genuine nephrosis. We are not yet in a position to offer a satisfactory explanation of this difference.

The question here arises as to the importance of the role played by changes of the serum's anti-hyaluronidase concentration in the mechanism of oedema formation. Most acceptable would seem to be the theory advanced in our above-quoted publication that hyaluronidase, which is otherwise inactive in the connective tissue, becomes increasingly active when the inhibitor level of the serum goes down and depolymerizes the hyaluronic acid contained in the connective tissue: this loosens the subcutaneous connective tissue and increases its "water avidity" which in turn leeds to the development of oedema. This explanation is in good agreement with the clinical observation that the oedema of nephritic patients feels differently from, much softer and pastier than, that of cardiacs: at palpation it conveys the same impression as that of oedemas provoked by the local injection of hyaluronidase.

Among others, *we demonstrated in one of our earlier series of experiments* that hyaluronidase gave rise to a local retention of fluids, i. e. that it increased the "water avidity" of tissues. It is known that, under the action of hyaluronidase, no painful tension of the tissues is felt even if high amounts of fluids are subcutaneously injected. In practice, hyaluronidase was therefore employed with preference in the case of young children and infants where the lack of larger superficial veins used to cause serious difficulties whenever an urgent parenteral administration of a larger quantity of fluid became necessary (Hechter, Dopkeen and Yudell 1947). The subcutaneous introduction of solutions containing hyaluronidase is without doubt much easier than of those which do not contain it. Our own experiences show that if, for instance, physiological saline contains hyaluronidase it can be infused about five times as quickly as the same solution under the same pressure without hyaluronidase; if the solution is injected the syringe needs considerably less pressure if hyaluronidase has been added to the fluid. After having been introduced, fluids with hyaluronidase will spread over the subcutaneous connective tissue much quicker than those without. The skin will not become taut but feel soft and oedematous at the site of injection. However, when examining this spot and its surroundings, a few hours later we found them still unchanged, i.e. soft and oedematous. We had the impression that the presence of hyaluronidase not only had not accelerated absorption but that there remained even more fluid than would have been left without hyaluronidase. Such observations induced us to make experiments to find out whether the absorption of subcutaneously administered fluid and dissolved substances was actually enhanced by hyaluronidase — a fairly widespread assumption at that time (1949) — or if it was only diffusion in the tissues which was promoted by it.

TABLE 29

*Anti-hyaluronidase titre in renal oedema*

| N° | Diagnosis | AHU/ml | | NPN | Glomer filtr ml/min. | Erythr sedim rate | Alb. % | Glob % | A/G |
|---|---|---|---|---|---|---|---|---|---|
| | | normal | pathol | | | | | | |
| 1 | Acute nephritis | (20) | 15 | 37 | 70 | 60 | — | — | — |
| 2 | Acute nephritis | (23) | 11 | 14 | — | 32 | 3·2 | 1 6 | 2 0 |
| 3 | Acute nephritis | (23) | 20 | 32 | — | 60 | 3·5 | 2·4 | 1·4 |
| 4 | *Chronic nephr.* | *(15)* | *1* | *150* | *1* | *120* | — | — | — |
| 5 | Pseudonephrosis | (15) | 2 | 77 | 11 | 70 | 1·9 | 4 5 | 0 4 |
| 6 | Pseudonephrosis | (15) | 2 | 39 | 14 | 55 | — | — | — |
| 7 | Pseudonephrosis | (20) | 4 | 75 | 9 | 105 | 2 0 | 2 5 | 0 8 |
| 8 | Pseudonephrosis | (21) | 2 | 37 | 22 | 122 | 1·9 | 1·8 | 1·1 |
| 9 | Pseudonephrosis | (21) | 0 | 30 | 74 | 88 | 1 5 | 1·6 | 0 9 |
| 10 | Pseudenephrosis | (15) | 2 | 72 | 15 | 70 | 2 4 | 3 4 | 0·7 |
| 11 | Genuine nephrosis | (21) | 21 | 28 | — | 28 | 1 5 | 2 9 | 0 5 |
| 12 | Genuine nephrosis | (21) | 40 | — | — | 35 | 2·0 | 3 0 | 0 6 |
| *13* | *Genuine nephrosis* | *(21)* | *42* | — | — | *15* | *1·2* | *3 2* | *0 4* |

Distally from the knee joint, 100 ml of physiological saline were infiltrated into both hind leg of dogs· the solution administered to one hind leg contained, that introduced to the other did not contain, hyaluronidase. An hour later, the animals were sacrificed, both legs disarticulated at the knee joint and then weighed.

It was found that, on an average, of our six cases the amount of absorbed hyaluronidase-containing fluid was less by 31 ml than that of the solution to which no hyaluronidase had been added. For the sake of control, we amputated and weighed also the hind legs of a further 9 animals. A statistical evaluation of the results proved $p$ to be less than 1% and thus highly significant. As has been mentioned, the experiments in question also showed that the local administration of hyaluronidase to animals which had undergone plasmapheresis produced oedema even if they had not yet become oedematous.

*R. L. Webb (1952) claims that the swelling of limbs* subcutaneously infused with isotonic saline and sugar solution subsides quicker if the solution contains hyaluronidase. This, however, does not necessarily mean an increased rate of absorption since the phenomenon may equally well be due to an enhanced rate of diffusion in the connective tissue. Webb, too, emphasizes that the subcutaneous administration of non-electrolyte solutions to dehydrated patients suffering from salt deficiency leads to a further temporary decrease of circulating plasma volume and that this danger is only enhanced by the presence of hyaluronidase in the introduced solution.

TABLE 30

*Action of hyaluronidase on the absorption of s.c. injected physiological saline solution*

(weight of extremities in g)

| I | II | Diff. | R. | L. | Diff |
|---|---|---|---|---|---|
| 132 | 120 | 12 | 217 | 216 | 1 |
| 135 | 132 | 3 | 152 | 154 | —2 |
| 265 | 210 | 55 | 214 | 212 | 2 |
| 350 | 320 | 30 | 370 | 396 | —26 |
| 480 | 460 | 20 | 319 | 319 | 0 |
| 500 | 435 | 65 | 230 | 240 | —10 |
| — | — | — | 120 | 100 | 20 |
| — | — | — | 160 | 145 | 15 |
| — | — | — | 152 | 151 | 1 |
| Average: | | 31 | | | 0·1 |

I = Weight of lower extremity after subcutaneous injection of 100 ml of physiol. saline containing 20 viscosity-reducing units of hyaluronidase.
II = Weight of lower extremity after s.c. injection of 100 ml of physiol. saline.
R = Weight of right leg } (control).
L = Weight of left leg }

This view was further substantiated by the investigations performed by Abbot and collaborators (1952). It was demonstrated by them that — while under the action of hyaluronidase, subcutaneously infused glucose solution actually spreads over a more extended area — the usual concomitant phenomena of subcutaneous sugar infusions (haemoconcentration, reduction of circulating plasma, volume, etc.) were intensified and their appearance accelerated by the effect of hyaluronidase. That this was so is attributable to the fact that water and electrolytes migrate from the blood plasma into the subcutis at a quicker rate so that the danger of collapses becomes greater. In one of their cases, for instance, subcutaneous infusion of 2 litres of 5% glucose solution (to which hyaluronidase had been added) was followed within 315 minutes by a 31 per cent reduction of circulating plasma and a drop of blood pressure from 100 to 70 mm Hg. Therefore, far from proving that hyaluronidase accelerates the absorption of fluids, these investigations rather seem to point in the opposite direction.

Forbes et al. (1950) declare that the absorption of subcutaneously injected isotonic saline solution is facilitated by hyaluronidase. However, these authors employed an indirect method by observing the disappearance of radioactive sodium from the site of the injection and its appearance in the blood plasma. But this is surely not enough

to allow of conclusions regarding the absorption of water. Their results were due partly to the fact that hyaluronidase accelerated the diffusion of radioactive sodium into the subcutis and its consequent disappearance from the site of injection and partly to a quickened equilibration of the radioactive Na-ions between subcutis and blood plasma. This, however, has nothing to do with fluid absorption, it simply means that capillary permeability became higher, allowing a more rapid diffusion of Na-ions through the capillary walls (in both directions).

That hyaluronidase increases capillary permeability is generally known (see our own investigations described elsewhere, and also those of Elster and his co-workers 1949a, b). Increased permeability leads to the escape of protein from the capillaries, to a reduction of the effective colloid-osmotic pressure and to a decreased absorption of the fluid injected into the subcutis irrespective of whether it contains sugar or electrolyte.

All these investigations led, thus, to the conclusion that larger doses of hyalurionidase decidedly impede the absorption of fluids and may in certain circumstances even give rise to the development of oedema. The passage of subcutaneously administered dissolved molecules into the circulation is, on the other hand, undoubtedly promoted by hyaluronidase.

This was confirmed by our experiments (Földi, Rusznyák and Szabó 1949c) in which we observed the effect of hyaluronidase on the excretion of inulin administered subcutaneously to dogs.

[illegible] (according to body [illegible]

the injected solution contained also 10 viscosity-reducing units of hyaluronidase. Since inulin is excreted exclusively by the renal route and excretion is fairly rapid and, further, in view of the fact that the quantity of renally eliminated inulin is [illegible] observed in the [illegible] aneously [illegible] ir filtrate [illegible]

The results of 5 such experiments are assembled in Table 31.

TABLE 31

*Amount (in g) of inulin excreted in 2 hours*

| Control | With hyaluronidase |
|---|---|
| 0·71 | 1·12 |
| 0 79 | 1 36 |
| 0 61 | 1·39 |
| 0 44 | 1 06 |
| 0·50 | 0 80 |

It can be seen that hyaluronidase increases the rate of inulin excretion very considerably, on an average by 45 per cent.

To demonstrate the increase in the rate of absorption in a direct way we injected s.c. small amounts of inulin solution of a known concentration — partly with and partly without hyaluronidase — at various sites at the same time. Withdrawing the fluid after some time from the point of injection, we determined its inulin level. In one case, for example, a solution containing 2·3 per cent inulin had been injected into the subcutis of the left paw of a dog: withdrawing the fluid after 15 minutes we found in it an unchanged inulin concentration. We injected the same amount of inulin into the other paw but had added hyaluronidase to the solution: inulin concentration in the fluid withdrawn 15 minutes later was found to have dropped to 1·9 per cent. Inulin concentration of the injected solution amounted in another experiment to 5·2 per cent, it dropped in an hour to 3·9 per cent where the fluid contained also hyaluronidase and to 4·8 per cent in the control fluid.

It was pointed out in our publication that this phenomenon admitted of various explanations. One of them would be that the injected hyaluronidase increases capillary permeability and therefore promotes filtration from vessels to tissues so that inulin is diluted by the escaped fluid. The results of our experiments made us rather incline to the conclusion that hyaluronidase increases absorption through the capillaries directly. This concept seems no longer acceptable without reserve as it is rather difficult to distinguish between cause and effect. Does a local reduction of inulin concentration occur as a consequence of enhanced permeability or is the rate of absorption quicker because of a more rapid spread of the dissolved molecules in the subcutis which come, thus, into contact with more capillaries and a larger membrane surface? This concept would nowadays strike us as highly acceptable if we were not confronted with results which show that a change in the rate of diffusion is not necessarily a decisive factor in the absorption of inulin. We refer to our experiments in which we tried to find out directly how hyaluronidase influenced the passage of inulin into the lymph vessels. The experiments proved — as will be explained in more detail later in this work — that the access of subcutaneously administered inulin to the lymphatics was not significantly influenced by hyaluronidase.

Taking all factors into consideration we are led to the final conclusion that the observed phenomena are obviously the outcome of complex factors. Hyaluronidase promotes the diffusion of inulin in the connective tissue and thus its absorption by the blood (and not the lymph) capillaries; it increases the permeability of blood capillaries and so contributes to the local dilution of subcutaneously introduced inulin; the enzyme may moreover promote absorption in a direct manner, that is, facilitate the diffusion of molecules through the capillary walls into the blood plasma by increasing the capillary permeability. From this point of view it makes no difference whether

to allow of conclusions regarding the absorption of water. Their results were due partly to the fact that hyaluronidase accelerated the diffusion of radioactive sodium into the subcutis and its consequent disappearance from the site of injection and partly to a quickened equilibration of the radioactive Na-ions between subcutis and blood plasma. This, however, has nothing to do with fluid absorption, it simply means that capillary permeability became higher, allowing a more rapid diffusion of Na-ions through the capillary walls (in both directions).

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[illegible] 2 to 2 g of inulin (according to body [illegible]

performed endogenous creatinine-clearance test showed that the glomerular filtrate was not affected by the administration of hyaluronidase.

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increased capillary permeability is due to the action of hyaluronidase or that of some other agent contained in testicular extracts. However, all these processes result in the acceleration of the process of equilibration between subcutis and blood plasma.

Returning now to the question whether, under normal conditions, the hyaluronidase—hyaluronic acid system plays any role in the transportation of fluids in the connective tissue and, thus, in lymph formation, we must admit that an answer to this question is not so easy. It was shown in the course of our experiments referred to in the foregoing paragraphs that the retention of fluids in the connective tissues and the development of oedema are promoted by hyaluroni-

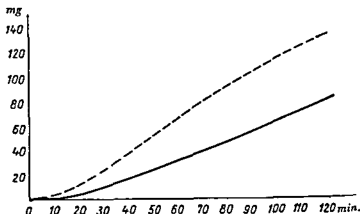



*Fig. 134.* Effect of hyaluronidase on the absorption of subcutaneously administered inulin

Dotted line renal excretion of inulin after injection of inulin + hyaluronidase; full line excretion of inulin injected without hyaluronidase

dase. Theoretically, such an effect may be developed in several ways, two of which seem to be of significance.

One would be that hyaluronidase — as has been shown — increases capillary permeability which leads to increased capillary filtration and increased protein content of the interstitial fluid; this, again, lessens absorption in the venous end of the capillaries (on account of reduced effective colloid-osmotic pressure) and results in the development of oedema. The second way would be that hyaluronidase provokes a loosening of the ground substance of connective tissue decreasing therewith tissue tension and pressure.

## ROLE OF TISSUE PRESSURE AND TISSUE RESISTANCE IN FILTRATION AND ABSORPTION

Tissue pressure used to be regarded as highly important in capillary fluid exchange. Even Starling mentions "back filtration", in the

development of which the hydrostatic pressure of extracapillary fluids plays an important role.

According to Landerer (1881), tissue pressure corresponds to two thirds of the "arterial capillary pressure", and he puts its value at 2-4 mm Hg. A direct measurement of tissue pressure is, however, rather difficult. The mean value of subcutaneous tissue pressure is estimated by Meyer and Holland (1933) at about 3 cm $H_2O$, whereas "intramuscular pressure" is somewhat higher being about double this. Similar results were reported by Beiglböck and Junk (1937), Burch and Sodeman (1937), as also by Wells, Youmans and Miller (1938). The last-named authors punctured the subcutis of human lower limbs with a fine capillary needle to which a manometer was attached. They found that the pressure in the loose subcutaneous connective tissue amounted to 0-8 cm $H_2O$ (with a mean value of 3 cm $H_2O$) and that it did not significantly rise even in venous congestion. McMaster (1947), employing a still more sensitive method, measured in the cutis of mouse ears an average "resistance" of 1·7 cm $H_2O$. In fact, the finer the method of measurement, the lower the connective-tissue pressure proved to be. On the other hand, Küchmeister (1953, 1954) — who adopted the technique of Beiglböck and Junk — registered in normal humans an average of 6·9 cm $H_2O$ for intramuscular and 2·4 cm $H_2O$ for subcutaneous tissue pressure. These values are hardly sufficient to be serious factors in the water metabolism of connective tissues and are surely not high enough to lead to the development of oedema through reduced tissue tension and resistance. We dissent from Küchmeister in this respect: he demonstrated that intramuscular pressure and "subcutaneous pressure" diminished in *oedematous patient. The decrease in the last-named* "pressure" amounts to 1·2 cm $H_2O$. Küchmeister (1953) attaches particular importance to the measurement of "resistance to flow". Following Meyer and Holland's technique (1933), he determines it by first measuring the "tissue pressure" and then ascertaining the amount of fluid which pours through the needle into the investigated tissue at a given (20 to 40 mm Hg) pressure. The amount of inpouring fluid would indicate the value of the "resistance to flow". Küchmeister claims that in disease resistance to flow weakens, both in the subcutaneous connective tissue and in the muscles.

We think that under normal conditions it is more justified to speak of "tissue resistance" instead of "tissue pressure". We believe — *and are in this respect in agreement with McMaster's theory* (1914a, b, c; 1946a, b) — that normal connective tissue contains no free fluid which has hydrostatic pressure. It was only the resistance which fluids have to overcome in order to gain access to the tissues which McMaster was able to determine by means of his micro-injection method. Locke and Tyrode solutions were taken up by the skin even at atmospheric pressure in his experiments; at atmospheric, and at very low positive (1 to 2 cm $H_2O$) pressure, fluid uptake was not

constant but intermittent, periodic, and not proportional to the pressure applied. If pressure rises above 4·5 cm $H_2O$, isotonic salt solutions will pour into the tissues at a constant rate although not even then will a correlation between pressure and water uptake become perceptible. A mixture of serum and Locke solution with pontamine blue (this dye increases capillary permeability and thus induces local oedema) does not stream into the skin at atmospheric pressure: to do so it needs a positive pressure of a few cm and then the stream will become continuous. The curve indicating the fluid uptake of connective tissues usually shows a sharp break at a pressure of 8·5 cm $H_2O$: thenceforward, a linear correlation between pressure and the amount of inflowing fluid will be demonstrable, irrespective of whether Locke solution, serum or pontamine blue solution is injected. We agree with McMaster in that it is at this pressure that the connective tissue ground substance is "broken up", "loosened", so that free fluid flow becomes possible.

We have seen that Meyer and Holland (1933), also Küchmeister (1953), determined "resistance to flow" by applying excessive pressures: they found that at such pressures there existed a linear relationship between the amount of fluid uptake and the height of pressure This led them to the conclusion that tissue fluid behaves as if it were in a capillary system of an order of magnitude in which flow is governed by the Hagen–Poiseuille law. We are, however, of the opinion that the method in question is not suitable for the determination of normal tissue resistance because high pressures prize the basic structure of the connective tissue asunder and, by doing so, give rise to an artificial system of slits which does not exist in normal circumstances.

It was reported by Wells and his associates (1938) that in venous congestion they found very high pressures in working muscles covered with fasciae. In cases of intensive contraction they registered intramuscular pressures over a range of 10 to 118 cm $H_2O$. These are, according to the authors, not enough to stop circulation in the affected muscle as the values in question are generally lower than the diastolic pressure. What they seem to forget is that pressure in the capillaries is in any case (even in maximal precapillary dilatation) dilatation inferior to the arterial diastolic pressure. Besides, tissue pressures of such magnitude would surely stop filtration, whereas we have seen that muscular activity promotes capillary filtration. We are, therefore, of the opinion that the only conclusion it is justified to draw from these investigations is that the method used is unsuitable for the measurement of "tissue pressure", a perfectly logical conclusion seeing that water in normal tissues does not move freely in a system of capillary clefts but occupies the surface of fibrils or the pores of molecular magnitude of the colloidal ground structure.

The interstitial space, on the other hand, actually contains free fluid in cases of oedema, and its pressure can be measured. Oedematous connective tissue does contain clefts since oedema — like fluid injected

under higher pressure in experimental conditions — breaks up or [illegible]

which, by loosening the basic structure of the connective tissue, allows pressure to remain unchanged while the amount of fluid increases. This concept seems to be substantiated by McMaster's observation that no fluid can be collected through a needle which is applied to the skin although the skin appears to be oedematous. Nor does tissue resistance increase in hyperaemia unassociated with oedema. In rapidly developing oedemas, however, especially in those induced — for instance — by a trauma of mouse ears used in his experiments, McMaster observed very high values of tissue resistance, values which might possibly check capillary circulation. As there exists free fluid in such cases it is possible to determine its pressure: McMaster found the pressure of oedematous fluid to be, in general, somewhat less than to "tissue resistance" (0·5 cm, on an average).

We believe, therefore, that a loosening of the colloidal ground substance, as caused by hyaluronidase, does not in normal circumstances lead to the development of oedema or the retention of fluids. The situation is quite different when there is free fluid in the subcutis or, generally speaking, in the connective tissue either because some fluid has been injected into it or because oedema has already developed [illegible]

is introduced into the oedematous tissue, fluid will escape therefrom, thus proving the presence of free fluid which extends, even disrupts, the ground substance. Hyaluronidase may, in such circumstances, indeed reduce tissue tension which, again, may lead — as a secondary consequence — to decreased fluid absorption after subcutaneous injections. The action of hyaluronidase in oedema consists — according to our concept — in the loosening of the basic structure of connective tissues and so the prevention of its "breaking"; this hypothesis would explain the differences existing between oedemas provoked by hyaluronidase and other — in the first place, rapidly developing — oedemas. The oedema-promoting action of hyaluronidase is, in our opinion, based on the effect of the factor which increases capillary permeability.

## EFFECT OF INCREASED CAPILLARY PERMEABILITY ON DIFFUSION

The confusion caused by the discovery that hyaluronidase preparations increased capillary permeability, characterized also the discussions which arose about the anti-hyaluronidase and permeability-

decreasing effect of the vitamin P. It was one of the present authors (Rusznyák) who, together with Szent-Györgyi (1936), first called attention to the fact that capillary permeability is lessened by certain flavone dyes. The investigations of Rusznyák and Benkő (1941) indicated that one was here probably dealing with a vitaminlike effect: in fact, the dyes in question have come to be referred to in the literature as vitamin "P" (permeability vitamin). Since then many publications have appeared which dealt not only with the practical application of vitamin P, but analysed also the mechanism of its action.

The exceedingly copious literature on this subject contains, of course, a good many contradictory data. It is claimed in several reports that, apart from their effect on capillary permeability, flavone derivatives diminish the permeability of the ground substance of the connective tissue, an action assumed to be based on the inhibition of the hyaluronidase effect (Martin and Beiler 1952; Martin 1953; Levitan 1949; Küchmeister 1954, and others).

However, Duran-Reynals (1954) seems to us to be perfectly justified in suggesting that the "anti-hyaluronidase" effect of diverse agents frequently does not consist in the inhibition of hyaluronidase: their action simply consists in strengthening the barrier function of the ground substance against certain materials whether hyaluronidase has previously been injected or not. He mentions, by way of examples, the ground substance of the skin of very young rabbits or of guinea pigs suffering from a deficiency of vitamin C: the spreading of fluids injected into such animals is very rapid in any case so that the addition of hyaluronidase to the fluid makes hardly any difference. Thus, in certain cases, ground substance behaves as if hyaluronidase had been administered. If spreading is hampered in such cases by some factor it need not necessarily be due to the inhibition of hyaluronidase. Changes in the rate of spreading can therefore arise not only through an inhibition of the action of hyaluronidase but also through some other mechanism. This concept is well-substantiated by the results of Levitan's investigations (1949). He suggests that though vitamin P undoubtedly combats the effect of hyaluronidase on the diffusion of injected fluids, it alone would probably inhibit their spreading quite as efficaciously. The inference made by Levitan in contradiction to earlier publications is that the action of vitamin P does not rely on hyaluronidase inhibition and that, moreover, it does not act on the capillary walls but on the connective tissue itself. It was, on the other hand, demonstrated by Elster (1949) that the rutin preparation used by Levitan, if administered in the form applied by him (65 mg suspended in physiological saline or dissolved in propylene glycol and injected intraperitoneally), provoked grave peritonitis in rats. Introduced intraperitoneally, propylene glycol alone also provokes local inflammation and haemoconcentration, and has the same effect as rutin solution. Perorally administered identical doses of rutin do not, however, pre-

vent the development of oedema elicited by intravenously injected hyaluronidase.

Not much more convincing were the results of Rodney and his associates (1950): they studied the hyaluronidase-inhibiting action of various flavonoids in vitro (by measuring their action on the viscosity-reducing and turbidity-reducing effect) of the enzyme and in vivo (by determining the rate of spreading with haemoglobin indicator).

In vitro, the major part of the best known flavone derivatives had no effect on the hyaluronic acid—hyaluronidase system. Ineffective were, for example, rutin, quercetin, hesperidin, homoeriodictyol, etc., while quercitrin and especially eriodictyol significantly lessened the effect of hyaluronidase. Hesperidin and homoeriodictyol were found to have no effect in vivo either. Rutin and quercetin had about the same effect. In-vitro experiments were also made with 1% tris (hydroxymethyl) aminomethane as solvent. Nearly all flavonoids inhibited the effect of hyaluronidase in this medium. We are of the opinion that experimental conditions (pH shift, high concentration, presence of foreign substances) were such as to make a correct evaluation of these results very uncertain. As regards the *in vivo* experiments of Rodney and co-workers, they have omitted to publish either methods or results with the necessary details. As far as can be gathered they administered the substance to guinea pigs intraperitoneally in a 1 % tris-(hydroxymethyl) aminomethane solution, and — under such conditions — most flavone derivatives diminished the effect of hyaluronidase. We think, however, that the objections made by Elster in connection with Levitan's investigations can be raised also against the experiments of Rodney and co-workers.

Küchmeister (1954) studied the effect of rutin in human beings with the cantharidin-wheal method and found that a preliminary treatment with hesperidinphosphate for 3 to 8 days decreased permeability very markedly, since the protein content of the wheal diminished, on an average, by about 50 per cent. He concludes that hesperidinphosphate probably acts as hyaluronidase inhibitor.

According to recent reports, it is possible to separate the two effects of hyaluronidase preparations, i.e. their action which promotes spreading in connective tissues from that which increases capillary permeability (Benditt et al. 1951): electrophoretic analysis shows that the latter is bound to another protein fraction (Tanos 1954; Turner 1954). These is actually nothing to prove whether vitamin P really affects the basic structure of the connective tissue or is only influencing the capillary permeability. Furthermore, it is impossible to tell which of the two afore-mentioned effects of hyaluronidase is inhibited by rutin.

Such considerations induced us (Szabó and Zsoldos 1951 unpublished, cit. Szabó 1954) to make experiments with a view to ascertaining how flavone derivatives influence spreading in the connective tissue.

Having shaved the abdomen of rabbits, 0·1 ml of physiological saline was injected into it by the intracutaneous route, India ink served as indicator. We observed the rate of spreading by measuring the size of stained area 20 min. after the injection. The experiment was repeated several days later during which rutin had been administered to the animals. The same procedure was followed in the investigation of the hyaluronidase effect, and the only difference was that, in these experiments, the i.c. introduced fluid contained also 40 U/ml of hyaluronidase. Rutin was given perorally in daily doses of 0·50 g/kg for 6 to 22 days, and we also studied the effect of intravenously administered citrin. Rabbits of 3 kg body weight received 0·40 g of this drug and spreading reaction was measured before its administration and 2 to 4 hours following it.

Results are assembled in Table 32. They show that neither rutin nor citrin affected the spreading of intracutaneously introduced fluid or the action of hyaluronidase on it. It is, therefore, probable that these substances affect merely the capillary walls, reduce their permeability and — as proved in the literature — inhibit the permeability-increasing effect of hyaluronidase. Essentially, these investigations supply further arguments in favour of the assumption that the action of hyaluronidase which increases capillary permeability, is different from that by which it promotes diffusion in the connective tissues and that each of these actions is due to a different mechanism. This is not without significance since it is affirmed by certain authors that the interendothelial cement of the capillary walls consists really of hyaluronic acid or some mucopolysaccharide related to it which is depolymerized by hyaluronidase in the same way as is the ground substance of the connective tissue. According to another theory, the capillary-permeability enhancing effect of hyaluronidase is due to its capacity to decompose hyaluronic acid situated in the perivascular basement membrane, or rather in the meshwork formed by the reticular fibres. This concept involves the hypothesis that these extracapillary or pericapillary

TABLE 32

*Effect of rutin on diffusion*

(size in sq mm of area stained by indicator)

| Before administration of rutin | | After administration of rutin | | Notes |
|---|---|---|---|---|
| Without enzyme | With enzyme | Without enzyme | With enzyme | |
| 348 | 745 | 365 | 540 | Rutin treatment: 1 week |
| 366 | 603 | 428 | 677 | Rutin treatment: 2 weeks |
| — | — | 442 | 704 | Rutin treatment: 3 weeks |
| 378 | 601 | 450 | 673 | Rutin treatment 2 weeks |
| 328 | 544 | 361 | 587 | Rutin treatment. 4 days |
| 330 | 565 | 375 | 592 | 2 hours after i. v. citrin |
| — | — | 368 | 503 | 4 hours after citrin |

connective-tissue formations play a decisive role in capillary permeability. Since, however, both the above-noted biochemical investigations and our own results have made it probable that the power of hyaluronidase preparations to increase capillary permeability cannot be attributed to the depolymerization of hyaluronic acid, this hypothesis cannot be true.

It has been noted that increase in capillary permeability and, in general, enhanced filtration raise the rate of spreading in connective tissues. Parsons and McMaster (1938b), for instance, studying the spreading of pontamin blue in the ear of mice with a micro method, found its rate to have been significantly increased by the dilatation of the precapillaries. The concept that augmented capillary filtration promotes spreading is well-substantiated by the experiments in which it was found that painting of the ear with xylene gave rise to increased diffusion. No such effect was observed if a considerable length of time elapsed between xylene treatment and examination. It is probable that (to use the term of Duran-Reynals 1942) "hyaluronate-inactive" diffusion factors (i. e. those which, in vitro, do not depolymerize hyaluronic acid) increase capillary permeability essentially through the liberation of histamine or in some similar way, and by doing so accelerate diffusion in the connective tissues. Anti-histamines, on the other hand, diminish the permeability of the connective tissue.

It was demonstrated b [illegible]
and antistine diminish [illegible]
the spreading effect o [illegible]
(Földi, Rusznyák and Szabó 1949b, 1950) confirmed this finding and [illegible]
exerts *in vivo* an anti-hyaluronidase effect which is independent from its anti-histamine action. *In vitro*, however, there is no such effect, antistine when applied *in vitro* did not influence the hyaluronidase, hyaluronic acid reaction.

All this lends weight to the supposition that substances which promote capillary permeability increase also the permeability of the connective tissue. Evans (1940), for example, showed that urethan and mercuric chloride enhance the permability of connective tissue as does histamine. In the course of our own experiments we found that arsenate — a substance known to increase capillary permeability (Unterberger and Böhm 1874; Magnus 1899; etc.) — also accelerated the diffusion in connective tissues.

We (Földi, Rusznyák, Szabó and Magyar 1954) used in these experiments the pentavalent arsenic salt — sodium arsenate — in concentrations of M/100 and studied diffusion in the connective tissues of rats and guinea pigs by means of our standard method.

Essentially, our standard method was to introduce intracutaneously 0·1 ml of fluid into the 24 hours previously shaved skin of the abdominal wall of the [illegible] of one and the same individual. Guinea pigs are given 4 injections each, on both sides of the shaved abdomen (the substance to be tested is administered on one the control fluid on the other side), on account of the smaller size of the available skin, surface only 2 to 3 i.c. injections can be performed on rats. As a rule, every determination was made in groups of 5 animals: the mean values of the individual deter- [illegible] comparatively short tim [illegible] makes measurements rat [illegible] we shall see later, the [illegible] globin which is — after [illegible] indicator, although many reports refer to its use in investigations of spreading effect. We observed that most of the commercially available India inks were precipitated by plasma proteins so that the coarser particles got stuck in even common filter papers. Seeing that connective tissues contain — according to their [illegible] — more or less plasma protein it seems to be [illegible] certain circumstances, undergo gross [illegible] why we used Congo red as indicator [illegible]

Our further procedure in these experiments was to determine the size of the indicator-stained area after the lapse of 2 hours: we measured its longest and shortest diameter and, regarding the area as an ellipse, calculated its surface. The index of action was the quotient obtained from a division of the stained area after application of the test substance by the control area. All results were statistically evaluated by Student's method. Before doing so, we ascertained that the results corresponded to the normal distribution so that the "$t$"-test could be applied.

Experiments performed with this method convinced us that spreading is facilitated by arsenate, if not strongly yet to a statistically still significant extent (Fig. 135). This effect of the arsenate was more marked when homologous serum instead of physiological saline solution had been injected.

It was shown by Zamboni and Cottafavi (1951) that, in rabbits, it is possible to prevent the permeability-increasing effect of the arsenate on the capillaries if antihistamines are administered. It was conceivable, therefore, that the spreading effect of arsenate was mediated also by histamine. This possibility induced us to start experiments with a view to determining how far antistine counteracts influence the action of arsenate.

Knowing that antistine possesses a pronounced anti-hyaluronidase effect and is able of itself to check spreading *in the connective tissue*, we performed two types of experiments whenever we wanted to study the effect of antistine. In the first kind of experiment 75 mg of antistine per kg of body weight was administered intramuscularly to the animals 10 to 15 minutes before the examination of spreading; then we injected intracutaneously the substance (in this case arsenate) together with the indicator dye into one side of the abdomen and the control fluid into the other. Results were read in the usual manner 2 hours later. In the second kind of experiment, the fluid to be tested was first injected intracutaneously and the area of its spreading determined 2 hours later; this done, antistine was administered through the intramuscular route and, 15 minutes thereafter, the same solution of the test substance injected intracutaneously anew; the diffusion of the second series was then measured 2 hours later. This second method seems to be most suitable for the determination of the effect which antistine exercizes on the spreading action of the

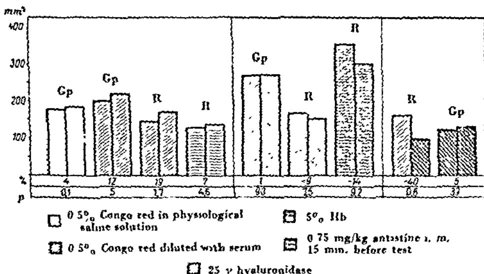



*Fig. 135.* Effect of sodium arsenate on the diffusion of dye in the connective tissue

Left column control tests Right column substance tested (M/100 $Na_2HAsO_4$). Gp = guinea pig. R = rat. mm² = size of skin surface dyed by indicator, % = percentage of change in dyed skin surface; p = probability according to Student

arsenic, yet, this method has the drawback that the time at which the intracutaneous injections are performed affects the results. The technique in question requires that the test animals be tied up for the duration of 2 hours, and it was observed that the permeability of connective tissues decreased even spontaneously after a certain time. We established this phenomenon by measuring the area of the spreading with our standard method in control animals and repeating the determination in the same animals 2 hours later. The stained area was found to be significantly smaller (11 to 13 per cent) at the second determination. This means that, under the given conditions, we cannot tell whether a certain substance does or does not inhibit diffusion unless the decrease in the size of the stained area is significantly greater than that observed to occur spontaneously.

As a matter of fact, antistine reduced spreading by 27 to 34 per cent (Fig. 136). It is therefore justifiable to claim that the spreading effect

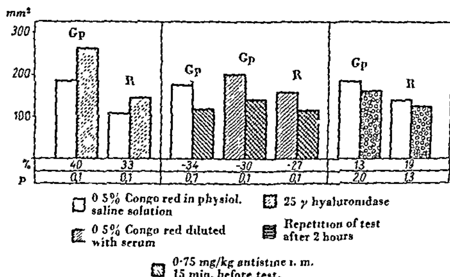



*Fig. 136.* Effect of hyaluronidase and antistine on the diffusion of dye in the connective tissue

Left column control tests Right column substance tested (M/100 $Na_2HAsO_4$), Gp = guinea pig, R = rat, mm² = size of skin surface dyed by indicator, % = percentage of change in dyed skin surface, p = probability according to Student

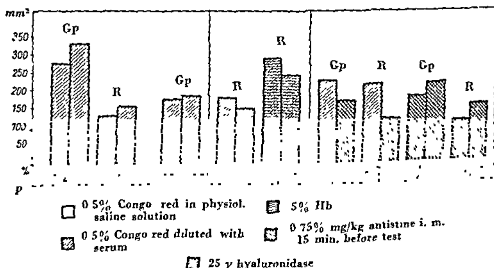



*Fig. 137.* Effect of monoiodoacetic acid on the diffusion of dye in the connective tissue

Left column control. Right column substance tested (M/1000 monoiodoacetic acid in tests 3 and 9, M/10 monoiodoacetic acid in all other tests) For further keys see Fig 135

of arsenic can be inhibited by antistine, and further that antistine reduces diffusion augmented by arsenate to a greater extent (—40%) than normal diffusion observed in the same species in the same manner (—27%). Thus, we can say that the diffusion-promoting effect of the arsenate is in all probability due to the action of histamine.

We further found that, apart from arsenate, monoiodoacetic acid, another well-known enzyme inhibitor, also promoted spreading in the connective tissue (Fig. 137). A concentration of M/100 increases diffusion significantly (by 18 to 20 per cent). Applied in a concentration of M/1000, the effect is no longer significant (+5%, $p = 0.22$). It is presumably not by liberating histamine that monoiodoacetic acid pro-

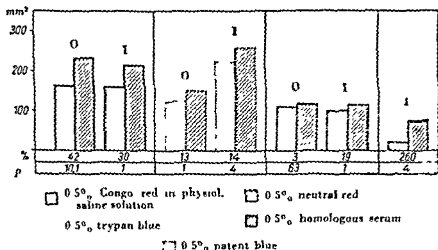



*Fig 138* Effect of homologous serum on the diffusion of various dyes in the connective tissue of guinea pigs

O = measured on the outside surface of the skin of living animals I = measured on the inside surface of the skin of dead animals

motes diffusion, but we think that, essentially, one is dealing also here with an increase of capillary permeability which is due to a direct capillary toxic effect.

Increased capillary permeability and the formation of oedema lead thus — as is clear from the literature quoted and proved also by our experiments — to accelerated diffusion in the connective tissues. Increased permability enables protein-containing fluid to gain access to the interstitial space. As it cannot be absorbed by the blood capillaries, distends and loosens the ground substance of the connective tissue and so facilitates diffusion. If this is really the case the same result should be obtained by creating similar conditions artificially through the injection of fluid However, intracutaneously introduced water is

readily absorbed through the blood capillaries but not if the injected fluid contains colloid. Proteins are not absorbed by the blood capillaries. Besides, proteins in the interstitial space reduce the effective colloid-osmotic pressure in the capillaries since the difference between the respective colloid-osmotic pressures of the extracapillary and intracapillary fluid become less, so that — locally and temporally, until the protein is carried away by the lymphatics — the rate of filtration increases in comparison with that of absorption. We deemed it necessary, therefore, to find out how spreading, i.e. diffusion of fluid in the connective tissues is influenced by the presence of colloidal molecules.

Experiments were performed with our above-described standard method: we injected the dyestuff in physiological saline solution into one side of the shaved abdomen of the animals, and in 0·1 ml of homologous serum into the other side. The size of the stained area was then determined 2 hours later. It has been pointed out in the foregoing that we had certain objections to the use of Congo red we therefore repeated the experiments with other dyes (patent blue, trypan blue) Some dyes have the disadvantage that it is rather difficult to determine the exact boundaries of the stained area· these are either blurred or the dye, e g. neutral red, fails to stain at the pH-values prevailing in the tissues. For this reason in some of the experiments the animals were killed and — after removing the skin from the abdominal wall — we made the necessary measurements on its inner surface. When neutral red was used we had to place the skin in an N/10 hydrochloric-acid solution since this dye must be developed with acid to become visible.

When Congo red or trypan blue were applied we found that the size of the stained area was significantly enlarged by the addition of serum; this increase in size manifested itself irrespective of whether the measurements were performed on the skin of the living animal or on the inner surface of the abdominal skin after the animals had been sacrificed. No significant difference was perceptible with the first method when patent blue was used as the boundaries of the stained area were so indistinct and dispersion so pronounced as to make measurements inaccurate. If, however, the second method was employed, i.e. if the measurement was made on the inner surface of the abdominal skin, even patent blue showed that serum proteins promoted diffusion.

## *FIXATION IN THE CONNECTIVE TISSUE AND SPREADING*

During these experiments the suspicion arose that the spreading effect of serum proteins was not really due to their effect upon the ground structure of the connective tissues but that they influenced the dye itself which was used as indicator, and that they influenced the fixation of the dye to the fibres and the ground substance of the connective tissues. Such suspicion seemed to be well-substantiated by our observation that basic neutral red, administered intracutaneously in physiological saline, almost entirely failed to spread. This

can be seen also by a comparison of areas stained by neutral red, administered without protein, with areas stained by other dyes (e.g. Congo red, patent blue, trypan blue). It shows that the spreading of neutral red is much slower than that of acid dyes and that, on the other hand, the diffusion of neutral red is much more vigorously affected by serum than that of the other stains. This may be due to a strong fixation of the basic neutral red to the ground substance of the connective tissue and to the fact that this fixation is prevented or weakened by serum proteins.

However, not only basic neutral red but also the other (acid) vital stains used by us may become strongly attached to the ground substance of the connective tissue: here, too, the presence of protein may make a difference. Congo red, trypan blue and patent blue are likewise adsorbed on and diffuse together with serum albumin. This induced us to use these dyes in a number of serial experiments as indicators of the spreading of serum proteins in the connective tissue. In this respect it is Congo red which is absorbed most intensively: 16 mol. of this dye are bound by 1 mol. of serum albumin, while adsorption is considerably weaker in the case of trypan blue and weakest in that of patent blue.

That dyes diffuse together with the plasma proteins to which they are fixed was further confirmed by the well-known investigations of Bennhold (1938) in which he studied the diffusion in gelatin of both protein-bound and free dyes. We have repeated and extended his experiments. We found that, in agreement with Bennhold's findings, Congo red — which failed to diffuse into the gelatin when dissolved in water or physiological saline — diffused into it together with the protein (true, at a rather slow rate) if we placed the dye on the gelatin together with serum or albumin. Since the molecular weight of Congo red in diluted sodium-chloride solution amounts, according to Svedberg, to about 8000 to 9000 (the molecules of Congo red aggregate to form a colloidal solution; the molecular weight of Congo red, computable from the chemical formula, is 652) and since Congo red attached to albumin must have a molecular weight at least as great as serum albumin, i.e. about 70 000, it is safe to assume that there exist also other factors (e g. electric charge, pH, etc.) beside the degree of dispersity on which the diffusion of Congo red into the gelatin depends.

Bennhold states that *it* is characteristic of dyes which are adsorbed by protein that their *rate of diffusion is equalized by the addition of protein.* This would mean that Congo red — *which, of itself, does not diffuse into the gelatin* — will spread in the same manner (*according to our observations, to a depth of about 4 to 5 cm in 72 hours*) as any other diffusible stain, *e. g.* methyl orange which spreads to a depth of several cm during the same time without protein but diffuses with the same velocity as Congo red if bound to protein.

Our experiments made it clear that serum has no effect on the diffusion of patent blue and trypan blue if they are employed in concentrations as used by us (0·5%): the dyes spread into the gelatin to a depth of several cm during the time of observation whether they had been dissolved in serum or physiological saline. This would mean that the greatest part of the dyes is free and does not become adsorbed to serum albumin in such concentrations. That this is so was shown when the two dyes were placed upon the gelatin in considerably thinner (0·05 to 0·01%) solutions: they no longer migrated into the gelatin quickly and freely but slowly and only together with the serum. We also made experiments in which the method of ultrafiltration was used: these, too, convinced us that both patent blue and trypan blue were mostly free in the concentration of 0·5 per cent, i. e. that they passed through the pores of the cellophane membrane of the ultrafilter which retains serum proteins almost quantitatively. The conclusion we are justified in drawing from these experimental results is that the diffusion of patent blue and trypan blue in the connective tissue was not essentially influenced by their adsorption to protein since both dyes were in the concentrations applied mostly free and unadsorbed in our experiments.

It has been pointed out above that the diffusion of acid vital stains might be strongly affected by their possible adsorption to the ground substance or the fibres of the connective tissues. This concept has attaine [illegible] -ch-kow [illegible] 36). Also [illegible] all over the body not later than 10 minutes after the intravenous administration of trypan blue and that the collagenous, reticular and elastic fibres had everywhere adsorbed the dye much earlier than it could be taken up by the reticuloendothelial cells and the histiocytes of the connective tissue through their colloidopexial action. We therefore, made experiments with a view to ascertaining how the fixation of dyes to subcutaneous connective tissue was influenced by serum.

We (Szabó and Koltay 1954 cit. Szabó 1954) removed a flap of skin from the abdomen of rats, cut the removed flaps into approximately equal pieces and weighed them: the weight of the pieces varied generally between 150 and 200 mg. We then [illegible]

It was found that, although the dyes dissolved in physiological saline had intensively stained the subcutaneous connective tissue, the staining was but superficial. After removing the layer that had been in immediate contact with the dye we saw that the deeper layers were unstained. Congo red and trypan blue dissolved in serum stained the corium to hardly any extent, staining by neutral red was also very

weak, whereas patent blue stained the inner surface of the skin pieces uniformly and intensively irrespective of whether it had been dissolved in saline or serum. Table 33 shows these results: taking the pieces of skin out of the dye solutions after an hour, we determined the dye content of the solution which showed the percentage of dye taken up by the skin during the one-hour incubation.

It has been known since Schade's experiments (Schade and Menschel 1923) that water is taken up by the connective tissue also in isotonic solutions. The tissues (liver, kidney, spleen, etc.) are, according to Opie and Rothbard (1953a, b), not isotonic with blood plasma; there exists no osmotic equilibrium between the living cells and the tissue fluid: the maintenance of the "tonicity" and ion composition of the cells depends on active enzymatic processes. As regards connective tissue, Opie and Rothbard's results are somewhat equivocal. While, after death, water is taken up osmotically by parenchymal organs in vitro (i.e. after the cessation of metabolism), the water uptake of the connective tissues depends (according to Opie) quite as much on the concentration of colloid as it does in the case of a 5—10—20% gelatine block. It is in any case sure — as has been proved by Opie and Rothbard's investigations — that water is quite markedly taken up by rat corium from a 0·15 mol. NaCl-solution. So the idea arose that the dye might diffuse into the corium together with the water and that swelling was prevented by the presence of protein so that no uptake of dye could result. This theory seems *a priori* untenable since it fails to account for the observed differences between the various dyes. We, nevertheless, performed certain experiments in order to observe the water uptake of the skin pieces. It was found that, if placed in physiological NaCl-solution, the skin flaps absorbed fluid to the extent of 30 per cent of their original weight and that the presence of protein (homologous serum diluted to 50%) exercised no significant effect on the water uptake. The absorbed fluid was not more than about 4 to 5 per cent of the entire solution in which the skin was incubated, while the amount of dye taken up varied between 20 and 30 per cent. The amount of absorbed fluid does not rise even if no dye is taken up by the corium (e.g. Congo red and trypan blue dissolved in serum). Uptake of dye is, therefore, quite independent of that of fluid. It should be noted that the swelling of the corium is not significantly affected by an even comparatively high concentration of protein in the surrounding fluid (3 to 4 per cent) which contradicts Schade's concept that water uptake is here due to the osmotic or oncotic pressure of the proteins (Table 33).

It has been noted that Opie and Rothbard found a far-reaching analogy between the water uptake of the connective tissue and that of gelatin. We have also noted that Bennhold observed essential differences between protein-containing and protein-free dye solutions in respect of their diffusion into gelatin blocks. In order to study this analogy between connective tissue and gelatin, we investigated the

TABLE 33

| | Physiol saline sol | | | | | | | Serum | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Weight of skin | | D mg | D % | V % | Conc. mg % | Ad-sorb. % | Weight of skin | | D mg | D % | V % | Conc. mg % | Ad-sorb % |
| | before | after | | | | | | before | after | | | | | |
| Congo red 10 mg % | 158 | 200 | 42 | 27 | 4·2 | 6·7 | 33 | 198 | 258 | 60 | 23 | 6·0 | 9·7 | 3 |
| | 177 | 212 | 35 | 20 | 3·5 | 7·1 | 29 | 184 | 236 | 52 | 28 | 5·2 | 10·0 | 0 |
| | 167 | 210 | 43 | 26 | 4·3 | 7·1 | 29 | 132 | 169 | 37 | 28 | 3·7 | 10·0 | 0 |
| | 137 | 176 | 39 | 28 | 3·9 | 7·1 | 29 | 185 | 225 | 40 | 21 | 4·0 | 9·8 | 2 |
| | 192 | 262 | 70 | 36 | 7·0 | 7·5 | 25 | 155 | 200 | 45 | 29 | 4·5 | 10·1 | —1 |
| Average: | 168 | 212 | 45 | 27 | 4·5 | 7·1 | 29 | 171 | 218 | 47 | 26 | 4·7 | 9·9 | 1 |
| Trypan blue 10 mg % | 205 | 261 | 56 | 27 | 5·6 | 8·0 | 20 | 180 | 234 | 54 | 30 | 5·4 | 10·0 | 0 |
| | 165 | 216 | 51 | 31 | 5·1 | 8·2 | 18 | 149 | 211 | 72 | 48 | 7·2 | 9·8 | 2 |
| | 215 | 277 | 52 | 24 | 5·2 | 7·6 | 24 | 205 | 268 | 63 | 31 | 6·3 | 9·8 | 2 |
| | 201 | 271 | 70 | 35 | 7·0 | 8·2 | 18 | 163 | 207 | 44 | 27 | 4·4 | 10·0 | 0 |
| | 157 | 210 | 53 | 34 | 5·3 | 8·2 | 18 | 164 | 214 | 50 | 30 | 5·0 | 9·8 | 2 |
| Average: | 189 | 247 | 56 | 30 | 5·6 | 8·0 | 20 | 172 | 229 | 56 | 33 | 5·6 | 9·9 | 1 |
| Patent blue 10 mg % | 211 | 271 | 60 | 28 | 6·0 | 5·9 | 41 | 166 | 220 | 54 | 32 | 5·4 | 7·2 | 28 |
| | 138 | 179 | 41 | 30 | 4·1 | 7·1 | 29 | 163 | 201 | 39 | 26 | 3·9 | 7·0 | 30 |
| | 134 | 170 | 36 | 27 | 3·6 | 7·4 | 26 | 148 | 176 | 28 | 19 | 2·8 | 7·5 | 25 |
| | 166 | 218 | 62 | 31 | 5·2 | 7·2 | 28 | 112 | 145 | 33 | 29 | 3·3 | 7·5 | 25 |
| | 172 | 222 | 50 | 26 | 5·0 | 7·2 | 28 | 152 | 188 | 36 | 24 | 3·6 | 7·5 | 25 |
| Average: | 164 | 212 | 48 | 28 | 4·8 | 7·0 | 30 | 148 | 186 | 38 | 26 | 3·8 | 7·3 | 27 |
| Neutral red 10 mg % | 137 | 194 | 57 | 41 | 5·7 | 6·7 | 33 | 158 | 194 | 36 | 22 | 3·6 | 8·5 | 15 |
| | 228 | 297 | 69 | 30 | 6·9 | 5·6 | 44 | 158 | 195 | 37 | 23 | 3·7 | 8·1 | 19 |
| | 167 | 215 | 48 | 29 | 4·8 | 6·0 | 40 | 125 | 162 | 37 | 29 | 3·7 | 8·1 | 19 |
| | 143 | 193 | 50 | 35 | 5·0 | 6·6 | 34 | 90 | 110 | 20 | 23 | 2·0 | 8·4 | 16 |
| | 127 | 182 | 55 | 40 | 5·5 | 6·6 | 34 | 175 | 225 | 50 | 28 | 5·0 | 8·1 | 19 |
| Average: | 160 | 216 | 56 | 35 | 5·6 | 6·3 | 37 | 141 | 177 | 36 | 25 | 3·6 | 8·2 | 18 |

water uptake of gelatin lamellas with the same method as was employed in connection with the dyes fixed by corium.

To obtain the necessary gelatin block we applied Bennhold's technique, i. e. we prepared a 5%-gelatin solution and adjusted its pH to 7·4 by means of buffer. Having poured the solution into a test tube it was left to dry and congeal. The rigid gelatin column was then removed from the test tube, cut into disks of 2 to 3 mm thickness; these were placed into the dye solutions and incubated there for an hour like the skin pieces (Table 33). The 5%-gelatin solution, used in these experiments, failed to swell in a 0·15 mol. NaCl-solution. (According to Opie, corium behaves in respect to water uptake like a 10%-gelatin solution.)

The behaviour of gelatin in phisiological saline solution with regard to dye absorption was found to be essentially the same as that of corium. All of the four stains were taken up from the saline solution and bound by gelatin to approximately the same extent as by corium. On the other hand, *gelatin failed to take up trypan blue and Congo red from the serum solution*, and only an insignificantly small amount of neutral red was absorbed. It was only patent blue which was taken up by gelatin to approximately the same extent from both saline and serum. That, under the given experimental *conditions, one was dealing in this case with superficial adsorption* and not diffusion, became clear when the uppermost layer of the disks was removed: the inner layers of the gelatin were completely unaffected by Congo red, trypan blue or neutral red. Only when patent blue had been applied, could a deeper diffusion of the dye into *the gelatin during the time of the experiment be observed*. We do not want to suggest that absolutely no diffusion takes place in the case of the other dyes: what we want to emphasize is that in our experiments fixation of dye occurred through its adsorption to the surface of, and not through its diffusion into, the gelatin. Diffusion is a considerably longer process: *dyes placed upon the gelatin require 24 to 48* hours to spread to a depth of a few mm, while the whole duration of our fixation experiments was no longer than an hour.

These investigations thus gave evidence to show that acid vital stains and also basic neutral red, brought into contact with gelatin *blocks and connective tissues, are strongly attached to their surface*. Binding fails to occur if the dyes were previously adsorbed to protein (serum albumin). This superficial adsorption is independent of the diffusibility of any particular dyestuff, for Congo red *which — in itself —* does not diffuse into gelatin is fixed not less than trypan blue *which is diffusible enough and whose fixation to protein rather tends*

NOTE TO TABLE 33

*Dye and fluid uptake by rat corium during one-hour incubation.* Incubation fluid 1 ml of physiological saline and homologous serum. Key: Weight of skin before-after = weight of the incubated pieces prior and posterior to incubation; (in mg). D mg = *increase in weight (mg)*. D % = *percentage of increase in weight*; V % = amount of absorbed fluid in per cents of original volume; Conc. mg % = concentration of dye in fluid after incubation; Adsorb. % = percentage of original amount of dyestuff in solution taken up by the test substance

TABLE 34

*Fluid and dye uptake of gelatin disks during one-hour incubation in physiol. saline and serum*

| | Physiological saline sol | | | | | | | Serum | |
|---|---|---|---|---|---|---|---|---|---|
| | Weight before | Weight after | D mg | D % | V % | Conc mg % | Adsorb % | Conc mg % | Adsorb % |
| Congo red 10 mg % | 107 | 129 | 22 | 20 | +2·2 | 8·2 | 18 | 1·07 | —7 |
| | 124 | 121 | —3 | —2 | —0·3 | 8·2 | 18 | 1·06 | —6 |
| | 148 | 122 | —26 | —18 | —2·6 | 8·0 | 20 | 1·07 | —7 |
| | 105 | 125 | 20 | 19 | +1·9 | 8·4 | 16 | 1·05 | —5 |
| Average: | 121 | 124 | 3 | 3 | 0·3 | 8·2 | 18 | 1·06 | —6 |
| Trypan blue 10 mg % | 157 | 175 | 18 | 11 | 1·8 | 6·2 | 38 | 1·03 | —3 |
| | 159 | 178 | 19 | 12 | 1·9 | 6·2 | 38 | 1·04 | —4 |
| | 161 | 144 | —17 | —10 | —1·7 | 6·2 | 38 | 1·04 | —4 |
| | 144 | 145 | 1 | 1 | 0·1 | 6·2 | 38 | 1·07 | —7 |
| Average: | 155 | 160 | 5 | 3 | 0·5 | 6·2 | 38 | 1·04 | —4 |
| Patent blue 10 mg % | 141 | 140 | —1 | —1 | —0·1 | 6·8 | 32 | 8·1 | 19 |
| | 157 | 230 | 73 | 46 | 7·3 | 7·4 | 26 | 8·3 | 17 |
| | 134 | 157 | 23 | 17 | 2·3 | 6·7 | 33 | 7·8 | 22 |
| | 142 | 135 | —7 | —5 | —0·7 | 7·4 | 26 | 8·0 | 20 |
| Average | 141 | 165 | 24 | 17 | 2·4 | 7·1 | 29 | 8·0 | 20 |
| Neutral red 10 mg % | 159 | 138 | —21 | —13 | —2·1 | 6·4 | 36 | 9·1 | 9 |
| | 164 | 167 | 3 | 2 | 0·3 | 7·3 | 27 | 9·0 | 10 |
| | 154 | 136 | —18 | —12 | —1·8 | 7·1 | 29 | 9·0 | 10 |
| | 168 | 143 | —25 | —15 | —2·5 | 7·2 | 28 | 9·1 | 9 |
| Average: | 161 | 146 | —15 | —10 | —1·5 | 7·0 | 30 | 9·05 | 9·5 |

Key: see Table 33, (p. 367)

to check diffusion. It is not merely in respect of swelling (Opie and Rothbard 1953a, b) but also in that of dye-adsorption that gelatin behaves like collagenous connective tissue. Collagenous fibres are capable of adsorbing dyes to their surface, a fact which has been proved also by the *in vivo* experiments of Anitschkow, McMaster and other authors. The dye-adsorbing properties of the connective tissue have to *be taken into account in any case, not only under experimental conditions* in connection with the diffusion of indicator dyes but also from the viewpoint of the physiological processes of metabolism occurring in the organism. That the connective tissue is capable of storing certain substances is a suggestion that has repeatedly been advanced in the literature of the last decades. Let us refer in this connection just to the problem of "dry salt retention" (Korányi 1930). Our own observations point to the probability that the fibres or the ground substance of connective tissues are able to bind certain substances so that concentration becomes higher on their surface than in the surrounding fluid. We are not in a position accurately to point out the substances in the organism to which this statement applies; the fact itself must in any case be taken into account when diffusion in the connective tissues and the passage of different substances from the blood path to the lymph vessels via connective tissue are investigated.

The results of the discussed experiments are surely such as may give rise to doubts concerning the correctness of our above supposition that diffusion in the connective tissue is promoted by protein and that we are dealing here with the tendency of colloids to cause local water retention and give rise to the development of oedemas. The diffusion-promoting action of proteins may be also explained by postulating a *process of adsorption-desorption. Dyes adsorbed to protein are not so* easily attached to the fibres of the connective tissues: therefore, they spread with the protein-containing fluid to a greater distance until the dyes become desorbed from the protein and adsorbed to the connective tissue fibres. However, not even this concept explains everything. The concentration of trypan blue as used in our diffusion experiments (0·5%) was such as the dye was surely no longer bound quantitatively to protein; our experiments with ultrafiltration and diffusion into gelatin gave sufficient evidence to show that the major part of the dye was in a free state under the given experimental conditions. This notwithstanding, the addition of protein was seen to promote also the spread of trypan blue very considerably.

Whether colloids really increase the permeability of the connective tissue cannot, therefore, be decided unless the experiments are repeated with other colloids. We have, for instance, observed (Szabó and Magyar 1954) that diffusion is facilitated by polyvinpyrrolidone (PVP) (periston, kollidon) quite as much as by serum albumin: a 3% solution of PVP (molecular weight, about 80 000) increased spreading in the subcutan connective tissue by 10 per cent. Experiments with PVP are, in point of fact, not suited for deciding our problem since synthetic

colloid adsorbs acid and basic vital stains just as much as does serum albumin (Bennhold 1951; Schubert 1951). PVP, like serum albumin, facilitates the diffusion of Congo red into gelatin: it has an "embatic effect" (Weese and Scholtan 1951) and even liberates dyes that have been taken up by the cells, it "washes the cells" (Schubert 1951). What we actually wanted to find out was how the action of hyaluronidase was affected by a colloid which is closely similar to albumin as regards its dye-absorbing and "transporting" capacity, one which fixes the indicator dye in the same manner as albumin. And this the more so

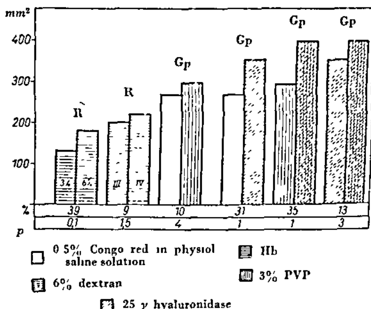



*Fig. 139* Effect of colloids on the diffusion of dye. For further keys see Fig 135

as, according to evidence cited in the literature, serum contains numerous specific and aspecific anti-hyaluronidase factor. We think that the question may be put also *in the following form: is serum not* able to inhibit the action of hyaluronidase alone by virtue of its physico-chemical colloidal properties? We found that the by 31 per cent increase of spreading achieved with our standard method by the addition of 25 micrograms of testicular extract-hyaluronidase remained unchanged when the injected fluid contained also 3% PVP (increase, 35%[illegible]read-promoting effect of PVP remained likewise unchan[illegible] by another colloid, dextran, which is used as plasma substitute like PVP (Szabó, and Magyar 1954). Spreading was increased by 24 per cent when a 6%

solution of dextran had been added to the injection fluid. This seems to be rather important since the dextran used in our experiments had the same dispersity as serum albumin, and the increase of diffusion, too, was approximately the same as that induced by homologous serum. However, as far as is known, dextran does not adsorb the dyestuffs. This would mean that, besides their adsorptive effect, colloids promote spreading by their mere presence.

It follows from the foregoing that the spreading effect of colloids must be due to their colloid-osmotic pressure in the tissues so that it may be expected to increase hand in hand with an increase in the colloid-osmotic pressure of the introduced solution. We instituted two kinds of experiments with a view to confirming this assumption.

Haemoglobin solution instead of dye was used in the first series of experiments. Employing our standard method, we injected a solution of 6% haemoglobin into one side of the abdomen of the test animals and the same solution, diluted by physiological saline to contain 3% haemoglobin, into the other side.

The fluid with the higher concentration of haemoglobin spread over a significantly larger area (increase, 39%!) than that with the lower concentration. This experiment showed at the same time that haemoglobin cannot be regarded as a perfectly indifferent indicator in investigations concerning spreading as — by virtue of its own colloid-osmotic pressure — it changes the permeability of tissues.

Likewise dextran, with Congo red for indicator, was used in the second series of experiments. Concentration of dextran was the same (6%) on both sides of the abdomen but we compared the effect of two dextran fractions with different molecular weights (fraction III—VI had an average mol. weight of about 60 000, fraction II—III one of about 150 000). We found that, compared with the fraction of higher mol. weight which had a lower dispersity, spreading was significantly more increased (on an average, by 9%) by the fraction of lower mol. weight which had a higher dispersity and — consequently — a higher specific colloid-osmotic pressure.

The results of our investigations seem, therefore, to prove that, while the spreading effect of plasma proteins as observed in the experiments with dye indicators may partly be due to phenomena of adsorption and desorption, the presence of colloid enhances the permeability of connective tissue in any case. If, therefore, proteins gain access to the connective tissue — be it in consequence of increased capillary permeability or for any other reason — they will promote diffusion: increase in the extracapillary colloid-osmotic pressure causes a parallel increase in the spreading of the proteins themselves (see haemoglobin experiments), while — quite independently — a simultaneous acceleration of the diffusion of other dissolved substances will also occur (see dextran—Congo red experiments). Such effect must be independent of the hyaluronic acid—hyaluronidase system: if the injected fluid contains hyaluronidase, the spreading effect of the colloids is simply added to the former.

## HUMORAL AND NEURAL FACTORS IN THE REGULATION OF CONNECTIVE-TISSUE PERMEABILITY

The permeability of connective tissues is regulated by the nervous system and the action of hormones. Especially detailed investigations have been made into the effect exercized by the hypophyseal-adreno-cortical system on the hyaluronidase—hyaluronic acid reaction.

As early as 1940 Weinstein demonstrated that spreading in the connective tissues was diminished by anterior pituitary extract; posterior pituitary extract has the same effect (Favilli 1939). We (Szabó 1954), too, found occasion to observe the diffusion-inhibiting effect of the posterior pituitary extract (pituitrin). That adrenocortical extract exerts a similar effect was shown by Menkin (1940), and his findings were checked and confirmed by subsequent experiments (Opsahl 1949a, b). Again, adrenalectomy is followed by an increased permeability of the connective tissue (Opsahl 1949b). This effect apparently depends less on mineralocorticoids since desoxycorticosterone acetate (DOCA) [illegible] on the other ha[illegible]eading effect of [illegible]imulation of the adrenal cortex has a similar effect. Diffusion is diminished and the action of hyaluronidase is checked, for instance, by the administration of adrenocorticotropic hormone (Vogt 1944; Long and Fry 1945; Selye 1946; Lurie 1950) or by a mobilization of the cortical hormone induced in some other way (adrenaline, morphine, subcutaneously *administered formalin, high temperature, cold* (Favilli 1939; Opsahl 1949a; Cahen and Grainer 1944; Zeckwer 1947; Shuman and Finestone 1950; Birke 1953). It should be borne in mind that the inhibition of spreading in these cases is not necessarily due to anti-hyaluronidase effect, since many of these substances are capable of inhibiting diffusion in the connective tissues independently of any enzymatic action. Essentially, their mode of action is, as has been mentioned, by inducing a change in the ground substance of the connective tissues.

However, the mechanism of this hormonal action is neither simple nor clear. It has been observed that a protracted treatment with ACTH, DOCA, cortisone, adrenocortical extract and progesterone tends to increase the permeability of connective tissues and the effect of hyaluronidase (Hayes and Bridgeman 1951; Favilli 1939; Birke 1953). Corticosteroids perform, according to Hayes, Reed and Baker (1950), their action in two ways. While, in acute experiments, steroids in the circulation have an anti-hyaluronidase effect, prolonged local or parenteral pre-treatment induces a change in the structure of the ground substance of the *connective tissues and so promotes spreading*. This might explain the contradictions between experimental results.

Tanos, Kelemen and Soltész (1953) suggest that pharmacological anti-hyaluronidase effects are largely of an indirect nature and

dependent on the presence of the adrenocortical system. It is possible that, to some extent, a mobilization of the active principles of the adrenal cortex is responsible for the anti-hyaluronidase action of salicylates described by Guerra (1946). It seems, however, that — besides the adrenal cortex — in some cases the medulla too plays a certain role in the development of the anti-hyaluronidase effect.

A great many substances have been found in the course of years to neutralize the action of hyaluronidase in vivo or in vitro, and to diminish the permeability of connective tissues. The effect of hyaluronidase is principally inhibited by other mucopolysaccharides, e. g. heparin, chondroitin sulphuric acid, gastric mucin, etc. (McClean 1942). That this is so is presumably due a mechanism of competitive inhibition, and our own investigations (Bagdy, Földi, Gerendás, Rusznyák and Szabó 1950), too, point in this direction.

These in vitro experiments supplied evidence to show that the thrombin-inactivating action of heparin is significantly impeded by hyaluronidase; the degree of inhibition becomes higher if heparin has previously been incubated with hyaluronidase for a number of hours. Inhibition of heparin by hyaluronidase manifests itself also in clotting induced by thrombin.

It can be seen from Table 35 that the time of clotting is prolonged if oxalate plasma is made to coagulate by means of thrombin (1) and if heparin is then added (2) to the system. If, however, heparin and hyaluronidase are added together (3, 4, 5, 6), clotting times will become shorter, a good indication of the antiheparin effect of hyaluronidase.

TABLE 35

*Demonstration of hyaluronidase-heparin antagonism in an experiment of clotting with thrombin*

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Plasma | 0·1 ml | 0·1 ml | 0·1 ml | 0·1 ml | 0·1 ml | 0·1 ml |
| Dist. water | 0·2 ml | 0·1 ml | — | — | — | — |
| Heparin 20 mg/ml | — | — | 0·1 ml | 0·1 ml | 0·1 ml | 0·1 ml |
| Hyase 55 U/ml | — | — | 0·1 ml | 0·1 ml | 0·1 ml | 0·1 ml |
| Thrombin 0·1 ml | — | — | 0·5 mg/ml | 1 mg/ml | 2 mg/ml | 4 mg/ml |
| Clotting time | 30 sec. | 58 sec | 47 sec | 44 sec. | 40 sec. | 33 sec. |

Thus, these experiments justify the assumption that the antagonism between hyaluronidase and heparin may be of a competitive nature; still more probable seems to us the assumption that the enzyme

becomes attached to the heparin: the latter is structurally very similar to the substrate, hyaluronic acid, so that heparin counteracts the effect of the enzyme on the hyaluronic acid.

*We are, in this connection, essentially in agreement with McClean's* views (1942). It was recently demonstrated by Alburn and Whitney (1954) that the action of hyaluronidase follows Menten's equation, i.e. that a complex of substrate and enzyme arises and that, thus, the action of heparin is really based on competitive inhibition. The anti-hyaluronidase effect of various hyaluronic-acid derivatives which are intensively fixed by the enzyme but are not depolymerized by hyaluronidase admits of a similar interpretation (Follett 1948; Hadidian and Pirie 1948; Pantlitshko and Kaiser 1951; etc.). A similar effect is probably exercized also by other sulphur-containing polysaccharides (Pantlitshko and Kaiser 1951).

In addition, permeability of the connective tissues is reduced by various phenol derivates (Calesnick and Beutner 1949) and antihistaminic substances (Mayer and Kull 1957). We succeeded in establishing the fact (Földi, Rusznyák and Szabó 1950) that the effect of the latter is not attributable to antihistamine action: these substances possess *in vivo* also a direct anti-hyaluronidase effect, although antistine, for instance, does not influence *in vitro* the viscosity-reducing effect of hyaluronidase on the hyaluronic-acid solution. Also a vast number of other organic substances of low and high molecular weight have been demonstrated as possessing marked anti-hyaluronidase effect, and it is impossible to quote them individually. Reference may be made to the reports of Hahn and Fekete (1953), Diczfalussy *et al.* (1953) and the comprehensive survey of Mathews and Dorfman (1954).

X-rays, on the other hand, induce a pronounced increase in the permeability of connective tissues (Wald and Varterész 1947, 1948; Kisselew 1951). This effect, too, may be caused on a change in the state of the hyaluronic acid situated in the ground substance of the connective tissues (Edgerley 1952, 1953). It is, however, quite possible that increased permeability of the connective tissue is — partly at least — due in this case to a liberation of histamine elicited by the irradiation (Forfota and Karády 1937).

Claude demonstrated in 1935 that azoproteins had a pronounced diffusion-promoting effect. Diffusion is further promoted by several reducing substances, e.g. vitamin C (Robertson and co-workers 1941), which depolymerize hyaluronic acid *in vitro*, also (Duran-Reynals 1942).

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nective tissues. It is for this reason that we attach great significance to a recent work of Kiss and Láng (1951) in which it is shown that the collagenous fibres of connective tissue are innervated by the autonomic nervous system.

Studying the autonomic [illegible] genous fibres of [illegible] and ligament of th. [illegible] would argue in favour of the conce[illegible] [illegible]ctional state of collagenous fibres and connective tissue is in fact directly affected by neural influences which, in turn, *might* affect the interstitial transport of water and dissolved substances.

Researches in this direction have hitherto [illegible] Absorption of intracutaneous [illegible] died by Hopf (1937) [illegible] the innervation, and by Sz[illegible] the completely denervated skin of guinea pigs. Both authors employed the method of McClure and Aldrich (1923) and observed a shortening of "resorption time". *We think the mere fact that the protuberance caused by the intracutaneous injection becomes impalpable after some time does not conclusively indicate the actual absorption of the injected fluid: it merely shows that it has spread in the skin.* Therefore, the method described by Aldrich and McClure should be regarded [illegible] perfect technique of spreading [illegible] ments of Hopf and Szontá[illegible] absorption but spreading [illegible] is facilitated by denervation.

The results of Berde's investigations (1949) seem to lend support to our arguments. After denervating with Mansfeld's [illegible] circular patches of skin on the [illegible] into the [illegible] and was [illegible] result: denervat[illegible] [illegible]ption of the phosphate, and no signif[illegible] could be observed between absorption from the denervated and that from the innervated area. We interpret this finding in the sense that the effect of denervation consists most probably in the mere acceleration of spreading.

*It would be, however, rather difficult to define* the mechanism through which denervation achieves this effect. It might be assumed that one is here not dealing with an effect upon the connective tissue itself and that the interruption of vascular innervation, a reduction of the tone of the vessels, provoke alterations in capillary circulation which give rise to increased diffusion. Yet, we are not inclined to accept this as the sole explanation of the phenomenon. The experiments of Harris and his associates (1952) supplied evidence to show that the rate at which intracutaneously introduced radioactive *sodium is*

absorbed depends on skin temperature. Aware that temperature of the skin is governed by the amount of blood streaming through it in unit time, i. e. by the width of the capillaries, the tone of the precapillaries, the results would permit the conclusion that the absorption of saline solution, injected into the connective tissue, is facilitated by accelerated blood circulation. Buchanan, Walls and Williams (1954) observed that the administration of trafuril — a rubefacient ointment which reddens the skin and raises its temperature — vigorously quickened the absorption of radioactive sodium. We, on the other hand (Szabó and Magyar, unpublished experiments), found that trafuril failed to accelerate the spreading, in rat skin, of Congo red dissolved in serum.

We see, that, while denervation promotes the diffusion of fluids in the skin, it fails to influence the absorption of injected electrolyte ions. Again, hyperaemia of the skin promotes the absorption of electrolytes but fails to influence diffusion. All this leads to the conclusion that increased spreading induced by denervation cannot be explained [illegible] rbance in vascular inner- [illegible] we are really confronted in this case with a direct effect on the connective tissues.

## EFFECT OF METABOLIC POISONS ON THE CONNECTIVE-TISSUE PERMEABILITY

*The results discussed in the foregoing paragraphs make it obvious that the permeability of connective tissues, the diffusion of water and* [illegible] a merely passive pro- [illegible] a close similarity to [illegible] lloidal systems, it has been shown that diverse physiological and pharmacological actions may cause profound alterations in the permeability of connective tissues. Such alterations are obviously of enzymatic origin (e.g. hyaluronidase effect) or provoked by a change in the structure or the ground substance of the connective tissues. This consideration induced us to make investigations into the effect exercized by metabolic and ferment poisons on permeability.

We have mentioned in the foregoing that our experiments regarding the effect of arsenate and monoiodoacetic acid led us to the conclusion that these substances increase capillary permeability and, by doing so, promote diffusion in the connective tissue. As regards monoiodoacetic acid, it was found that besides its direct toxic effect on capillaries, it influences also the hyaluronidase—hyaluronic acid system in the connective tissue. [illegible] ulated diffu- [illegible] dose of M/1000, had no significant effect on diffusion but markedly

diminished the spreading effect of hyaluronidase. Such effect cannot be due either to a change in the fixation of the indicator (Congo red) to the connective-tissue fibres, or to an influencing of the anti-hyaluronidase effect of Congo red, seeing that the same results could be achieved with another indicator, namely *haemoglobin*.

M/1000 monoiodoacetic acid was found to inhibit the action of hyaluronidase in vitro also.

We performed our in vitro experiments with hyaluronidase examining its viscosity-reducing action of hyaluronic-acid solutions. This is the simplest of all known methods for the determination of hyaluronidase. First, unpurified hyaluronic acid was prepared by the extraction of human umbilical cord with distilled water and the precipitation of the extract by means of potassium acetate and saturated alcohol, from this hyaluronic acid we then prepared a solution which had a relative viscosity of about 4 (as compared with the M/6 acetate buffer of pH 6 used as solvent).

We made the viscosimetric determinations with Ostwald's viscosimeter at 37° C according to the method of McClean (1943). Dorfman and co-workers (1948a). Rodney and co-workers (1950) described a method for calculating the percentage inhibition in viscosimetric measurements with the aid of mathematical formulae. Our experiments convinced us, however, that these methods are unreliable because dispersion is wide in the quantitative evaluations and also because it is very difficult to reproduce the results in serial experiments. Although we computed the percentage hyaluronidase inhibition using viscosimetric values, nevertheless, our results are of an informatory character only, from which no further conclusions must be drawn. Alluring as it would seem to make comparisons between the results obtained in vivo and in vitro, we refrain from doing so, for we are quite aware of the errors inherent in both methods and are convinced that nothing but erroneous conclusions would result from such comparisons.

In vitro experiments made with these methods gave the result that the addition of M/1000 iodoacetate to the system completely paralyzed the viscosity-reducing action of hyaluronidase. Inhibition was complete, no matter which method of computation was employed. In this respect, our results are in perfect agreement with the earlier experiments of Glick and Kaufman (1950) as also with those of Chain and Duthie (1940).

We have noted that capillary permeability is enhanced by arsenate. Its effect is presumably due to the liberation of histamine: the spreading effect of hyaluronidase is, as has been proved by our experiments, hardly affected by it. It is conceivable that arsenate may — to a slight extent —really impede the action of hyaluronidase: this inhibitory effect is, however, more than compensated by the increase in capillary permeability to which it gives rise. The effect of hyaluronidase was actually observed to have been somewhat diminished in vitro through a treatment with M/1000 $Na_2HAsO_4$ but the uncertainty of the experimental method makes it very questionable whether one is justified in regarding this result as significant.

Further experiments showed that potassium cyanide in concentrations of M/100 and M/1000 diminished spreading in rats and guinea pigs very considerably. This inhibitory effect manifested itself irrespective of whether Congo red, patent blue or haemoglobin was used as indicator. Differences were greatest in guinea pigs with the use of

Congo red and patent blue dissolved in serum (—30 and —29%). In rats the degree of inhibition was 25 per cent whether Congo red dissolved in serum or 5% haemoglobin was used as indicator.

Potassium cyanide in a concentration of M/100 also counteracted the spreading effect of hyaluronidase. This should not be interpreted in the sense that cyanide has a direct local inhibitory effect on the [illegible] in order to counteract the effect of hyaluronidase do not exert a specific action on the enzyme itself. They quote, as example, salicylate which neutralizes hyaluronidase in very high concentrations only (Dorf-

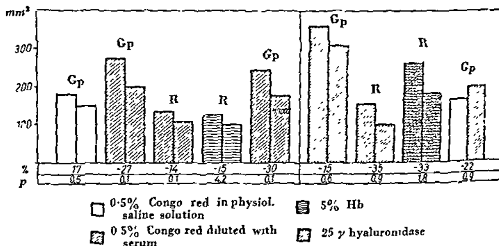



*Fig. 140.* Effect of potassium cyanide on dermal spreading
Left column control, right column: M/100 KCN (M/1000 KCN in test 5) For further keys see Fig 135

man and co-workers 1948b). Although cyanide and the other ferment poisons studied by us are effective in a concentration of M/1000 and even in lower concentrations it would be wrong to affirm with certainty that KCN acts directly on hyaluronidase. That caution in this respect is justified was proved by our next series of experiments which showed that cyanide was unable to impede the effect of hyaluronidase (25 γ, which corresponds to about one turbidity-reducing unit) even if applied in concentration of M/100: hyaluronidase still had a spreading effect if even to a lesser degree. It is, therefore, safe to conclude that the anti-spreading effect of potassium cyanide is not, or not exclusively, based on its power to neutralize hyaluronidase, a very important fact if we remember that the action of hyaluronidase was very markedly paralyzed by cyanide in our in vitro experiments (inhibition amounted to 50 per cent when determined by Dorfman's method, to 80 per cent

with the technique of Rodney et al.). Our results are in good harmony with those of Glick and Kaufman (1950) who observed *in vitro* an inhibition of 60 per cent with a concentration similar to ours.

Sodium fluoride, applied in a concentration of M/100, checked dermal spreading and it made no difference whether Congo red or haemoglobin served as indicator, nor whether the dye solution contained protein or not. The action of fluoride *in vivo* is probably not based on specific inhibition of hyaluronidase since the decrease in spreading (—19%) did not seem to depend on the presence of the enzyme. *In vitro*, fluoride impedes the action of hyaluronidase less than do either cyanide or monoiodoacetic acid, but the inhibitory effect is still demonstrable (40 per cent with Dorfman's and 24 per

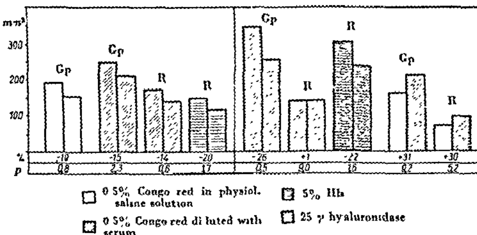



*Fig. 141.* Effect of sodium fluoride on dermal spreading
Left column control, right column M/100 NaF For further keys see Fig. 135

tent with Rodney's method). As in the case of cyanide a close connection between in vivo and in vitro effect seemed to be lacking. Robertson and his associates (1940), who preceded us in studying the effect of fluoride on hyaluronidase *in vitro*, found that fluoride did not affect the action of hyaluronidase. These authors used *Clostridium welchii*-mucinase; their results seem, however, to be of doubtful value seeing that they studied, among others, heparin and iodoacetate in the same series of experiments and found them to be ineffective, whereas repeated subsequent experiments by other workers have proved that both substances possess marked anti-hyaluronidase effect *in vitro* even in low concentrations.

Among all investigated substances there is only one, namely dinitrophenol, of which we can say with complete assurance that, in a concentration of M/10 000, it possesses anti-hyaluronidase effect *in vivo*.

Spreading was diminished by M/10 000 dinitrophenol alone to approximately the same extent as by the use of KCN in a concentration of M/1000 (—16 to —17 per cent; Fig. 142). If in addition to dinitrophenol hyaluronidase is added to the fluid to be injected, the spreading effect of the latter (25$\mu$g) is almost entirely paralyzed. On the other hand, we found that — [illegible]
of dinitrophenol [illegible]
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hyaluronidase effect also *in vitro*, but it should be noted that, relatively to the amount of hyaluronidase, they applied it in a considerably higher concentration than we so that their results are not comparable with

mm 300 200 100 R R R R R

% -16 -17 7 -29 -20

p 1 12 55 18 47

0·5% Congo red in physiol. saline solution; 25 $\gamma$ hyaluronidase; 5% Hb

*Fig. 142* Effect of dinitrophenol on dermal spreading

Left column control, right column M/10 000 DNP. For further keys see Fig. 135

those of our *in vitro* experiments. In view of the fact that we observed almost complete inhibition *in vivo* it must be said that here, too, a parallelism between in vivo and *in vitro* effects is lacking.

Finally, we also made experiments to find out how diffusion in the connective tissues behaves after death, i.e. when not only certain biochemical processes are impeded by ferment poisons but in a condition where, together with life, all such processes have actually ceased. Parsons and McMaster (1938b) showed that diffusion considerably decreased in the mouse-ear after the death of the animal. Hechter (1950) attaches great importance to investigations of this kind as superposed reactions can be eliminated in experiments on dead animals. Therefore, Humphrey and Jaques (1953) recommend that spreading effect in freshly killed guinea pigs be determined for the biological titration of hyaluronidase.

Investigating the spreading of physiological saline and protein-containing solutions in rats after the death of the animal we found

spreading to have been still quite markedly increased by serum 3 hours after death (22%) while such effect was no longer demonstrable after the lapse of 24 hours. A comparison of the absolute values of spread-area as measured on carcasses, with values obtained from other experiments makes it obvious that intracutaneously introduced fluids spread over a considerably smaller area in dead than in living animals. It was proved by Parsons and MacMaster (1938b) that movement promoted spreading; as a matter of fact, they regard movement as the only decisive factor. Our own experiments confirmed this finding insofar as dermal spreading was in fact considerably increased by an active movement of our test animals or a mild massage of the investigated areas. McMaster regards arterial pulsation as very important for the diffu-

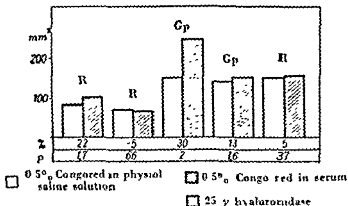



*Fig 143* Diffusion in the connective tissue after death Animals were passively moved in the last series of experiments. For further keys see Fig 135

sion in the connective tissues and the *lymph flow* in the small lymphatics. Parsons and McMaster (1938b) observed that intracutaneously administered dyes diffused at a quicker rate, gained access to and flowed in the small lymphatics more rapidly in amputated, isolatedly perfused mouse ears when the vessels were perfused with a pulsating stream than in experiments where the vessels were flooded with a steady, non-pulsating blood circulation at the same pressure.

How important movement is has been brought home to us in experiments in which we compared diffusion in immobile rat carcasses with spreading in carcasses in which artificial respiration was induced through a cannula inserted into the trachea. It was found (Fig. 143) that artificial respiration, i.e. imitation of a steady physiological movement, increased diffusion to a considerable extent.

Also the spreading effect of hyaluronidase was examined in dead rats According to reported data, this effect can be demonstrated even on pieces of skin removed from the animals and on the corpses

themselves. We found that spreading in 90-minute old rat carcasses was in fact very markedly increased (by 24%) by hyaluronidase; the effect became much less pronounced by the 24th hour after death; by this time the increase dropped from 24 to about 13 per cent. Such drop was probably due to the fact that, in the meantime, autolysis — and maybe also bacterial decomposition and putrefaction — had exercized their action on the enzyme substrate in the connective tissue.

The net result of all these experiments was the proof that various enzyme poisons are capable of significantly affecting the permeability of connective tissues. Even *in vitro* these poisons more or less inhibited the power of hyaluronidase to depolymerize hyaluronic acid. It would be attractive to explain the diffusion-inhibiting effect of enzyme poisons by the hypothesis that they paralyze the action of the hyaluronidase present in the connective tissues also under normal, i.e. physiological, conditions. However, the presence of hyaluronidase in the skin and the subcutaneous connective tissue has — as has been pointed out — never been proved with absolute certainty. Nor must we forget that no accurate parallelism between the *in vivo* and *in vitro* effect of enzyme poisons could be observed. Cyanide, for instance, impedes the action of the enzyme to a considerable extent *in vitro* and has hardly any such effect in vivo. Again, fluoride has a somewhat less marked anti-hyaluronidase effect *in vitro* and fails to affect the enzyme *in vivo* although, in themselves, both fluoride and cyanide retard dermal spreading to a considerable extent. Dinitrophenol checks diffusion and combats the action of hyaluronidase *in vivo* but, though applied in the same concentration, has hardly any effect *in vitro*. Monoiodoacetic acid which *in vitro* completely paralyzes the action of hyaluronidase, and arsenate which — although to a lesser extent — also possesses an anti-hyaluronidase effect reveal likewise significant differences between their respective behaviour *in vitro* and *in vivo*.

## DIFFERENCE BETWEEN HYALURONIDASE EFFECT *IN VITRO* AND *IN VIVO*

All these results seem to show that the action of enzyme poisons is not or not exclusively due to their influence on the reactions between hyaluronidase and hyaluronic acid. The literature contains numerous reports to show that there exists no close parallelism between hyaluronidase effect *in vivo* and *in vitro* Hobby, Dawson, Meyer and Chaffee (1941) were the first to investigate this subject thoroughly. True, McClean (1943) dissents from this concept: he claims the existence of a statistically high correlation between the diffusion-promotion, viscosity-reduction and mucin-clot-prevention of different enzyme preparations. The results published by him are, however, not too convincing, the ratios of the spreading to the viscosity-reducing effect vary between 44 and 156, those of the other *in vitro* method (mucin-clot-prevention)

to the spreading effect between 0·4 and 25. Hobby and his co-workers also pointed out that preparations which were inactive *in vitro* were, nevertheless, capable of promoting spreading *in vivo*; further, that serum-containing specific anti-hyaluronidase and paralyzing the action of the enzyme in vitro failed to do so *in vivo*. This problem has been discussed in many recent publications: in Hungary, Kelemen and co-workers (1953) concerned themselves with the subject.

It was in connection with Congo red that the problem seemed to us especially interesting and important. Ferraro et al. (1948) gave evidence to show that hyaluronidase effect is significantly inhibited by the parenteral introduction of Congo red. Experiments made by Tanos (1954) and further experiments performed by us made it evident that the hyaluronic acid depolymerizing action of hyaluronidase was completely inhibited by Congo red *in vitro*, while inhibition with the same concentration was far from complete *in vivo*.

It is suggested by Tanos that only one of the actions of hyaluronidase — that which depolymerizes hyaluronic acid — is paralyzed by Congo red, while the dye has no effect on its other action, that which increases capillary permeability and is, according to him, independent of the first-named action. Tanos made his suggestion on the evidence of experiments in which hyaluronidase of testicular origin was compared with that of bacterial origin. A rat-limb-oedema test (Kelemen, Iványi and Majoros 1951) furnished evidence that bacterial hyaluronidase (Hyason-Organon) contained 15 to 20 times less hyaluronate-inactive, permeability-promoting substance than testicular hyaluronidase although both kinds of the enzyme were applied in amounts of equal viscosity-reducing concentrations. By adding an adequate amount of Congo red to hyaluronidase prepared from testicular extract they could completely stop its depolymerizing effect without impairing its power to provoke oedema.

Referring to Hechter's (1950) finding that it is characteristic of hyaluronate-active, spreading factors that under their influence diffusion reaches almost its maximum within 5 to 15 minutes while — if provoked by hyaluronate-inactive, spreading factors — diffusion proceeds gradually for a longer time, Tanos started experiments in which Congo red and haemoglobin were used as indicators. He observed that the size of the stained area was uniformly increasing even after a longer time when Congo red was applied while hardly any increase could be perceived after the 15th minute when haemoglobin was used. He concluded that, in the presence of Congo red, solely the non-depolymerizing oedema-promoting substance was able to act. However, an inspection of Tanos's results (which he had kindly put at our disposal) failed to fully convince us of the justification of his claim, since we found the scattering in his results to be fairly great. Therefore, we (Szabó and Magyar 1954, cit. Szabó 1954) repeated the experiments in question. It can be seen from Figs. 144—147 and from Table 36 that the spreading effect of hyaluronidase is in fact damped in the presence of Congo red, yet, the phenomenon mentioned by Tanos cannot be observed. The experiments performed with enzymes of different origins (testicular-extract-Hyalase Richter and bacterial Hyason-Organon) yielded parallel curves so that there seems to be no justification for the claim that the presence of Congo red stops the effect of Hyason after the first 15 minutes and that this dye checks the spreading effect of the bacterial enzyme more promtly than that of the testicular extract. If, therefore, the assumptions of Hechter

and Tanos regarding the effect of the two enzymes are correct, there can be no question of solely the permeability-increasing effect remaining operative in the presence of Congo red.

We are, thus, of the opinion that there can be no doubt as to the existence of a discrepancy between the influence of Congo red on the hyaluronate-depolymerizing effect of hyaluronidase in vitro and its spreading effect *in vivo*, for a concentration which is sufficient to inhibit

TABLE 36

*Spreading effect of hyason and hyalurase on diffusion, with Congo red and haemoglobin as indicators*

(area in mm²)

| | | Hyason | Hyalurase |
|---|---|---|---|
| Congo red | control | 145 | 159 |
| | enzyme | 194 | 189 |
| | enzyme / control | 1.34 | 1 19 |
| Haemoglobin | control | 164 | 173 |
| | enzyme | 251 | 228 |
| | enzyme / control | 1 53 | 1 32 |

the action of hyaluronidase completely *in vitro* hardly affects it *in vitro*. This difference may, to some extent, be due to the fact that *in vitro* tests are more sensitive than the method of hyaluronic-acid depolymerization so that when hyaluronidase effect is no longer detectable with the latter method, an increase in dermal spreading may still occur in vivo (as a consequence of a still existing reaction between hyaluronidase and hyaluronic acid). It is, of course, also possible that the explanation of the discrepancy between behaviour *in vitro* and *in vivo* [illegible] nective tissues is not the [illegible] Our views regarding the [illegible] increasing action have been set forth in the foregoing. Hyaluronidase preparations may, however, have also other than hyaluronic-acid-depolymerizing and capillary-permeability-promoting effects which may, too, *influence* the permeability of *connective tissues*.

Most noteworthy in this respect are the investigations of Gersh and Catchpole (1949) who studied the effect of collagenase on the

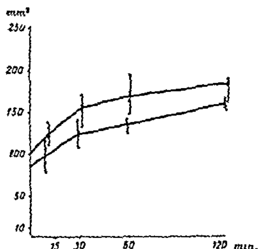



*Fig. 144.* Effect of hyalurase (Richter) on diffusion, with Congo red as indicator

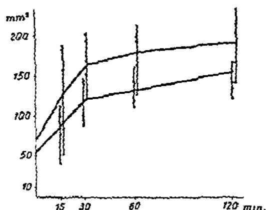



*Fig. 145* Effect of Hyason (Organon) on diffusion, with Congo red as indicator

Ordinate area (in mm²) stained by indicator, abscissa time of measurement Lower line control, upper line test with hyaluronidase

ground structure of substance tissues. Collagenase (*Clostridium-welchii* toxin) was described by Maschmann (1938) and later by Oakley and his associates (1946) as also by other authors. Collagenase seems not to affect the collagen itself since it does not disintegrate the fibres of connective tissues, but depolymerizes the mucopolysaccharides in their ground substance. *Clostridium-welchii* toxin is moreover known to increase the permeability of the connective tissue (McClean 1936).

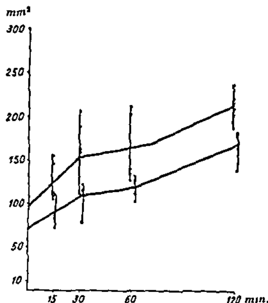



*Fig 146.* Effect of hyalurase on diffusion, with haemoglobin as indicator

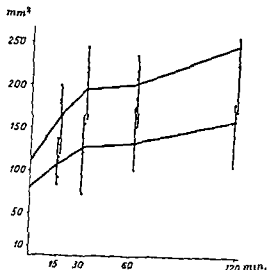



*Fig. 147.* Effect of hyason on diffusion, with haemoglobin as indicator

Ordinate: area (in mm²) stained by indicator, abscissa time of measurement. Lower line control, upper line: test with hyaluronidase

These authors compared *Cl.-welchii* toxin, testicular hyaluronidase and a purified mucinase preparation with regard to their spreading effect hyaluronic acid depolymerizing action and activity in the azocoll-test (the "azocoll test" was recommended by Oakley for the

measurement of collagenase activity); they found that their testicular hyaluronidase preparation which, on account of having been stored for several years, had lost its power to depolymerize hyaluronic acid was still able to decompose azocoll quite markedly and to increase (though not vigorously but still significantly) spreading in the connective tissue. Their purified mucinase preparation showed no collagenase activity but depolymerized hyaluronic acid quite strongly and exerted a marked spreading effect. *Cl.-welchii* collagenase, on the other hand, depolymerized both azocoll and hyaluronidase and seemed to possess a vigorous spreading effect. Histochemical experiments showed that all of these three substances dissolved the glycoproteins of the ground substance of the connective tissues if the latter were incubated with the enzyme for 20 hours.

TABLE 37

(after Gersh and Catchpole 1949)

| | Depolymerization of hyaluronic acid | Depolymerization of azocoll | Spreading effect | Glycoproteins in the ground substance of the skin |
|---|---|---|---|---|
| *Cl.-welchii* toxin | +++ | ++ | +++ | Decomposed after 20 hours |
| Testic. hyaluronidase | ± | ++ | + | |
| Purified mucinase | +++ | 0 | + | |

We, therefore, agree with the theory that the permeability of the connective-tissue ground structure is regulated not by one but by several enzymes. It is, however, doubtful whether any *in vitro* method of investigation concerning hyaluronidase or collagenase activity can supply information as to how these factors affect the functional state of the ground substance of the connective tissue and if they play any part in its physiological regulation at all. Let us not forget that the presence of hyaluronidase in the skin has never been reliably proved. Gersh and Catchpole examined also the collagenase activity of human tissues. Only tumor tissues were found to be markedly active, whereas normal skin, extracts of gastric mucosa and of renal tissue displayed no activity. These observations justify the supposition that hyaluronidase plays no part in the normal metabolism of connective-tissue polysaccharides and that the synthesis and decomposition of polysaccharides should be attributed to other enzymes (Mathews and Dorfman 1954).

Our own investigations, too, support the theory that — if enzyme poisons exert an inhibitory effect on any enzyme participating in the regulation of connective-tissue permeability — this enzyme is probably not hyaluronidase. Nor can we accept the concept of Tanos that one

is dealing here with an effect upon that factor which is responsible for increased capillary permeability. Most acceptable seems the assumption that, under physiological conditions, it is neither hyaluronidase nor collagenase but another — in its effect similar — enzyme which regulates the permeability of connective tissues. This theory would explain the discrepancy between *in vitro* and *in vivo* results and also the reason why ferment poisons reduce dermal spreading even in cases where they do not impair the spreading effect of hyaluronidase.

But this last phenomenon may perhaps admit of another interpretation also. It is quite possible that, instead of acting upon an enzyme present in the connective tissues and decomposing the polysaccharides of their ground substance, the ferment poisons exert a direct effect on the living ground substance or its metabolism. Advancing farther along this line of reasoning it seems justified to suppose that the spread of water and colloids in the connective tissues is, in fact, not or not exclusively based on a process of diffusion but that, in addition, some other transport mechanism associated with active energy consumption is at work. This hypothesis will become more probable than is apparent first sight if we take into account all the knowledge we have gathered in the last years about the permeability of cells.

We have seen that spreading is decreased by respiratory enzymes, especially by cyanide which paralyzes the cytochrome system. But a similar effect is exerted also by dinitrophenol which — while failing to affect oxygen uptake — impedes the formation of phosphates of high energy content by separating the processes of oxidation and phosphorylation. Disturbance in the decomposition of phosphoric-acid esters, too, decreases diffusion since fluoride also has a decided antipermeability effect. Iodoacetate, on the other hand, which paralyzes the metabolism of carbohydrates at an earlier phase than fluoride, exerts a marked spreading effect. (This effect is probably due to the fact that monoiodoacetic acid increases capillary permeability.)

Although our experiments with metabolic poisons are, thus, not conclusive enough to enable us to point with accuracy to the processes or enzymes which regulate the permeability of connective tissues, they at least justify the conclusion that disturbances in the tissues *or in the metabolism of the connective tissue are not* without influence on conditions regarding permeability.

Experiments with dead animals substantiate the correctness of this concept. We have noted that dermal spreading is slower after death than in living animals and that a quickening of the rate can be induced by artificial movements (e.g. artificial respiration). Yet, spreading in dead animals can never be raised as much as in living ani- [illegible] dermal spreading [illegible] 24-hour old rat [illegible] cent lower than [illegible] with different

enzyme poisons yielded approximately similar results. This may be interpreted to mean that, to this extent, diffusion in the connective tissue depends on vital phenomena, the functioning of metabolism (and circulation), while any further decrease in diffusion, as observed on dead animals, may be ascribed to lack of movement.

## FACTORS INFLUENCING SPREADING IN CONNECTIVE TISSUE

*Summary*

We have, in the foregoing, surveyed the factors which play, or may play, a role in regulating diffusion in connective tissues and in the transportation of water, dissolved colloids and crystalloid molecules to the lymph capillaries. It was found that also the permeability of the blood capillaries themselves influenced diffusion in the connective tissue. Of decisive importance for the permeability of connective tissues are the mucopolysaccharides situated in the ground substance and on the surface of the fibres. Spreading is vigorously promoted by enzymes which depolymerize these large colloidal molecules. It has further been noted that the effect of these various "diffusion factors" is far from uniform. We have also discussed the problem concerning the neurogenic and hormonal regulation of connective-tissue permeability.

It has been shown that — apart from hormonal factors — a number of other substances exert influence on the permeability of connective tissues. We discussed the pharmacological action of certain metabolic poisons more thoroughly and came to the conclusion that diffusion in the connective tissue is probably no mere passive process since metabolic poisons, save those which presumably act by increasing capillary permeability, reduce the permeability of connective tissues more or less significantly.

This hypothesis seemed to be substantiated by observations on dead animals. Our experiments proved the significance of movement for diffusion in the connective tissue and furnished, at the same time, arguments in favour of our assumption that metabolic processes may have some importance in the permeability of connective tissues.

Formation of oedema promotes tissue permeability and so does the presence of proteins in the connective tissue facilitate the spread of water and dissolved substances; we tried to prove that this is essentially due to increased colloid-osmotic pressure in the tissues.

Serum proteins (albumin, in particular) facilitate, moreover, the spreading of vital stains by the adsorption of dyes and by preventing dyestuffs from being bound by connective-tissue elements. This phenomenon must be taken as indicative of the fact that the fixation of certain substances to the structural elements of the connective tissue necessarily involves their spreading. That this is so was most

strikingly illustrated by the behaviour of neutral red: this basic dye became virtually *fixed at the site of injection.* We propose to discuss in the following the problem of this fixation which has a considerable significance in inflammatory processes.

## FIXATION IN INFLAMMATORY TISSUE

It was pointed out in the chapter on the increase of capillary permeability in connection with inflammation that colloidal molecules and particulate matter are accumulated in the tissues during inflammatory processes. We want, here, to content ourselves with a short reference to some of the pertinent reports.

Okuneff (1924) showed that, in local stimulus by high temperature, intravenously introduced vital stains became visible in the injured area of the skin. According to Kusnetzowsky (1925), intravenously [illegible] inflammatory area under [illegible] e. g. mustard oil. Kettle [illegible] ion had previously been provoked by various irritants in the skin of rabbits and mice — intravenously administered tubercle bacilli appeared in the inflamed area. Again, Bowman, Winternitz and Evans (1912) demonstrated that, in experimental tuberculosis, the tubercles were intensively stained by intravenously administered vital dyes (trypan blue and trypan red). Chesney, Turner and Halley (1928) found that spirochaetae intravenously or intratesticularly injected into rabbits, appeared very soon in wounds [illegible] in the area of the [illegible] taneous injectio[illegible] that it was in these abscesses that intravenously introduced Streptococcus viridans emerged. Similar observations were made also by Burrows (1932), Fox (1936), Menkin (1929, 1930) and others.

In point of fact, however, all that is proved by these observations is that capillary permeability is increased in inflammatory area. But it is not merely a question of colloids and corpuscular elements escaping from the capillaries more easily in the inflammatory area: it is undoubted that these substances are, moreover, bound by, and fixed to, the tissues. Pawlowsky demonstrated long ago (1909) that the entrance of intra-articularly injected staphylococci into the circulation is seriously delayed if inflammation has previously been induced in the joint. According to Issayeff (1894), resistance to intraperitoneally administered virulent microorganisms is temporarily enhanced by a previously provoked sterile peritonitis. Opie (1929), repeating the experiment several decades later, confirmed the finding of the Russian investigator and found that the passage of intraperitoneally injected haemolytic streptococci into the blood stream was impeded by sterile peritonitis provoked with aleuronate. Opie (1924) also established the fact that — while, under normal conditions, no inflammatory reaction

is released by subcutaneously administered foreign proteins, the previously sensitized animals in presence of which the blood can be demonstrated very promptly — the protein antigen is not absorbed but becomes fixed at the site of the injection and an inflammatory reaction at this point occurs (Arthus' phenomenon). Jancsó and Jancsó-Gábor (1952b) demonstrated with the aid of their special staining technique that the major part of administered proteins becomes fixed at the site of Arthus' phenomenon. The complex formed by antigens and antibodies largely accumulates in the reticulo-endothelial cells.

It was observed by Menkin (1929) that, in cases of inflammation induced by the subcutaneous administration of aleuronate or starch solution, trypan blue injected into the inflamed skin becomes locally fixed: the dye fails to spread, and does not enter the regional lymph vessels and lymph nodes even after a long time. His further investigations furnished evidence to show that also other substances — such as colloidal iron, graphite, bacteria and heterogeneous proteins — become fixed in the inflammatory area (Menkin 1930, 1931a, b).

With regard to the mechanism of the phenomenon at issue, it was stated by Menkin (1930) that a fibrinous network is formed in the inflammatory area which prevents the substance in question from spreading and entering the lymph vessels while, at the same time, thrombi arise in the lymphatics which impede lymph circulation (for further details see Menkin's monograph 1940b).

We are afraid that Menkin's interpretation of the process in question is too simple. We have pointed out in the preceding chapter that great importance attaches to the adsorption of various substances to the fibres and the ground substance of connective tissue. There can be no doubt that a change in the nature of the binding, a closer relationship between the structural elements of the connective tissue and the dyes, proteins or bacteria that had left the capillaries exercize such an effect on the phenomena at issue as must lead to a fixation of the said substance in the area of inflammation. A mechanism of this kind, too, is in our view involved in giving rise to the "fixation" observed inflammatory processes.

In the search for the mechanism of the reaction it was, for instance, proved by Drennan (1951) that an "acute watery oedema" arises whenever connective tissues are traumatized (chemically, mechanically, thermally, bacterially) and the mast cells pour their metachromatic substance into the injured area. The essential nature of the metachromatic granules in Ehrlich's mast cells still needs elucidation. There exist, in any case, many data which seem to prove that the granules in question consist of heparin (Holmgren and Wilander 1937; Jorpes 1946; Oliver, Bloom and Mangieri 1947; and others). Heparin is known to have a marked anti-hyaluronidase effect and to inhibit diffusion in connective tissues. Fixation in the area of inflammations is, thus, in our view a far-from-simple process in which also numerous other mechanisms than that postulated by Menkin may be involved.

# CHAPTER VIII

# ABSORPTION INTO LYMPH CAPILLARIES

To remove proteins and other colloidal substances that have gained access to the interstitial space is the task of the lymphatics. We have seen that another important task of lymphatics is the carrying away of interstitial fluid. Lymph vessels cannot transport fluid or proteins unless these substances — no matter how they have got from the blood capillaries into, say, the subcutaneous connective tissue, whether in a physiological way, through filtration or artificially through subcutaneous injection — first reach the walls of the lymph capillaries, i. e. spread in the interstitial spaces of connective tissue. The interstitial spreading of fluids and colloidal molecules as also the factors capable of influencing the permeability of connective tissue under physiological and pathological conditions were discussed in detail in the preceding chapter. We propose to deal, in the following, with the question of how fluid and dissolved molecules, once they have reached the wall of the lymph capillaries via the ground substance of the connective tissue, find their way into the lumen of the lymphatics.

Hudack and McMaster (1933), examining the small lymph vessels of the skin after intracutaneous injections of vital dyes, found that — however slight the lesion caused by the needle was — the dyestuff appeared in the small lymphatics instantly; a stained network of capillaries and small lymph vessels around the puncture became visible very promptly. Their conclusion was that even the finest cannulae must have damaged the lymphatics so that the dyestuff poured directly into them. This would mean that intracutaneous injections are, in fact, intralymphatic injections. However, McMaster (1947) is not quite consistent in this respect: his recent, above-discussed investigations have led him to the conclusion that pressure in the lymphatic capillaries is generally lower than "tissue resistance". In the course of his experiments on the mouse's ear he established the mean value of the "tissue resistance" at 1·9 cm water (result of 11 experiments) and the average intralymphatic pressure at 1·2 cm. Fluids are thus, according to the [illegible] the interstitial space to the capillaries. But these measurements are, to say the least, of a doubtful value. To begin with, it is rather obscure what in point of fact is "forced" into the lymph capillaries by the difference of pressure under normal conditions when there is no free fluid in the connective tissue. In cases, on the other hand, where the skin contained oedema fluid, McMaster found the average fluid pressure

to be 0·5 cm lower than the "tissue resistance" which makes it extremely probable that — when free fluid is actually present as it is, for instance, in the oedema or in the lumen of lymph capillaries — the method in question will yield lower values so that, in normal tissues, there seems to exist no difference of pressure between extra- and intracapillary fluids.

The situation is essentially different when free fluid is actually present in the connective tissue. It is known from McMaster's experiments that the pressure of the interstitial fluid may have a fairly high value and reach even a level of 20 cm water. Differences between tissue pressure and intra-lymph capillary pressure can be quite significant and facilitate a "filtration" of the fluid into the lymphatics. We want, in any case, to make it clear that — in our opinion — a tissue pressure superior to intralymphatic pressure cannot constitute the sole and decisive factor responsible for the absorption through lymphatic capillaries. Zhdanov (1952), for instance, emphasizes in his monograph on the lymph vascular system that the physiological and anatomical properties of the lymph capillaries, the fact that their permeability exceeds that of the blood vessels, a direct connection with the ground substance of the connective tissue, the possibility of strong fluctuations of calibres and the physiological activity of the lymph capillary endothelium are surely important factors of the absorption into the lymph capillaries. We want now to examine all possibilities arising in connection with the absorption into the lymph capillaries.

## STRUCTURE OF THE LYMPH-CAPILLARY WALL. PASSAGE OF CORPUSCULAR PARTICLES INTO THE LUMEN OF LYMPH CAPILLARIES

The morphological properties of the wall of lymph capillaries are certainly significant in connection with absorption through lymph vessels. Colloidal molecules, but also corpuscular particles, injected into the subcutis, appear in the efferent lymphatics within a few seconds, a phenomenon attributed by Hudack and McMaster to injury of the lymph vessels. The possibility was also considered that lymphatics had no continuous walls but were pierced by stomata and pores or that the end of the lymph capillaries was open so that they were in direct contact with the "tissue sap". However, morphological investigations made it clear already at the beginning of the century that the lymph capillaries were closed at their end (MacCallum 1903) and that their walls contained neither stomata nor any other kind of apertures.

But the question of whether in certain functional states there may not arise apertures in the lymph-capillary walls is still the subject of controversy. This idea was first advanced by Kolossow (1889) who thought that a change of pressure, a dilatation of the vessels might pull the endothelial cells apart giving thus rise to apertures between

the intercellular bridges. A number of later authors concerned themselves with this problem. Henry (1933), for instance, reported that the subcutaneous injection of India ink into the ear of rabbits was followed by the appearance of apertures in the lymph-capillary walls through which the granules of the dye were able to slip: such apertures were demonstrable even after the lapse of a day or two. Clark (1936) claims that an increase of pressure causes the lymphatics to distend and gives rise to the appearance of apertures in their walls which disappear with the return of normal conditions but may subsist for several days.

Like those of blood capillaries, the endothelial cells of lymph capillaries are connected by interendothelial cement that can be demonstrated by impregnation with silver. As in the case of blood capillaries, the idea arose that the composition of the cement in question might be identical with that of the ground substance of the connective tissue. However, no direct proof in this respect has been furnished.

Following the analogy presented by blood capillaries, a simple explanation of the passage of fluids and colloidal molecules into the lumen of lymphatics would be to assume that the interendothelial cement is provided with submicroscopic pores of molecular size through which water and dissolved molecules are diffused, "filtered", into the lymph vessels. The explanation becomes less simple in regard to the absorption of particulate matter. We have noted that certain authors postulated the existence of temporary stomata. Relying on the results of their functional investigations, Field and Drinker (1931a, b) refuse to accept this concept. Nor do they think it probable that it is by means of a lesion of the lymph capillaries that subcutaneously administered proteins gain access to the lymphatics. After introducing horse serum into the neck of dogs by the subcutaneous route, they had to wait four hours until, by gradual rise, the concentration of protein reached a certain level in the efferent lymph vessel. The injection of a fresh dose failed to cause a further increase in the protein level of the lymph although such increase ought to have occurred in a case of direct intralymphatic injection or if stomata in the wall of the lymph vessels had arisen. Drinker, in the last analysis, accepts the theory nevertheless that lymph capillaries are anatomically closed units which [illegible] conditions. He [illegible] movement, [illegible] the clefts [illegible] condition- [illegible] the passage of corpuscular elements into the lymph vessels.

Another possibility to be considered in connection with the absorption of corpuscular particles is that these particles and the larger colloidal molecules are phagocytozed by the endothelial cells of the lymph capillaries and so passed on to the lumen of the vessels. Drinker and Yoffey (1941) do not think that phagocytosis by the endothelium of lymph capillaries occurs under normal conditions. Repeated injection

of vital dyes into adult animals is followed by a pale and diffuse staining of the lymph vascular endothelium, but this phenomenon is in no way similar to the storage of granules in the Kupffer cells or other reticuloendothelial cells.

Wislocki (1916, 1917) found that lymph vascular endothelium was vigorously stained when frog larvae had been kept in a solution of trypan blue for a week. Clark and Clark (1918, 1919) state that symptoms of a phagocytosis of graphite and carmine particles in the lymph vascular walls are demonstrable on the transparent tail of frog larvae. It was observed by Karsner and Swanbeck (1920, 1921) that the intrapleural administration of lampblack to cats was followed by a phagocytosis of the introduced corpuscular elements in the endothelial cells of the immediately adjacent subpleural lymph vessels.

Commenting upon these reports, Drinker observes that what they essentially prove is just the fact that the lymph vascular endothelium of very young animals is capable of phagocytozing corpuscular elements in certain conditions, e.g. in cases of chronic irritation, while they fail to answer the question whether phagocytosis plays an important role in the entrance of particulate matter into lymph vessels. A survey of the pertinent literature favours the view that phagocytosis must not be regarded as an important factor, for none of the published reports proves that phagocytosis constitutes a normal physiological function of endothelial cells.

## PHAGOCYTOSIS OF PROTEINS BY LYMPHATIC ENDOTHELIUM

It follows from the above-quoted reports that, under certain conditions, corpuscular elements may be phagocytozed by the endothelial cells of lymphatic capillaries (although this phenomenon has no great significance from the point of view of capillary permeability). No reliable data are, however, available to prove that normal capillary endothelium is capable of phagocytozing colloidal molecules: reports on the subject are all based on the results of pathological-anatomical investigations.

Randerath (1948) reported, for example, that in cases of amyloid nephrosis paraprotein was stored in the endothelial cells of the renal lymph capillaries. A similar observation was made by Fresen (1942) who reported the storage of lipo-protein in lipoid nephrosis. The endothelial cells of the lymphatic capillaries turn, according to Fresen, into "froth cells" and "degenerate" in this manner.

Seeing that it is very difficult to distinguish even normal lymphatic capillaries and that both Randerath and Fresen examined only pathological material, we found it necessary to ascertain whether normal lymphatic endothelium also possessed the capacity of accumulating proteins.

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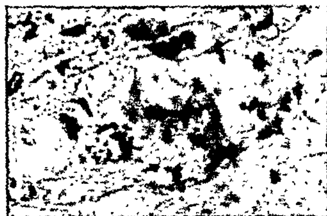


*Fig. 148.* General view of a large lymph path. Granules of suramin in the endothelium of the lymph vessel (black). Such granules are visible also in the interstitial space and the lymph



*Fig. 149.* Enlargement of Fig 148. Suramin easily-visible in endothelial cells. Very little lymph in the lumen of the vessel which, also contains suramin. The histiocytes of the interstitial tissue, moreover, outside of the endothelial layer, contain granules of suramin

In order to render protein visible, the technique described by Jancsó and Jancsó-Gábor (1952a, b) was employed in our experiments (Foldi, Jellinek, Rusznyák and Szabó 1954). It is known that Bayer 205 (germanin, suramin) is firmly fixed by plasma proteins. A histochemical method for the demonstration of the germanin-protein complex in the tissues has been elaborated by Jancsó and Jancsó-Gábor: it consists in the fixation and staining of small tissue pieces in May-Grünwald solution and their embedding in paraffin after dehydration in benzene. Thus treated, the germanin-protein complex stains dark violet and becomes clearly distinguishable in the sections.

Jancsó and his associates demonstrated that the germanin-protein complex was to be principally found in two types of cells: in the cells of the RES (in the histiocytes in particular) and in the kidney, in the cells of the proximal tubules. The experiments of Jancsó and his co-workers seem to prove that the physiological function of histiocytes consists in the uptake of proteins from the interstitital tissue. The appearance of the germanin-protein complex in the proximal renal tubules indicates the path covered by proteins filtered under physiological conditions and absorbed by the cells of the tubules.

After anaesthetizing rats with ether, we ligated their left ureter and injected 150 mg/kg of germanin into the peritoneum after the lapse of 48 hours. The ligature of the ureter had the purpose of dilating the renal lymph vessels so as to make them visible in histological sections. The animals were sacrificed at 2, 4, 6 and 9 days, respectively, after the injection and their kidneys treated according to the — somewhat modified — technique of Jancsó and Jancsó-Gábor.

We are able to confirm Jancsó's finding that the proximal renal tubules are filled with the germanin-protein complex. In addition, we observed the protein complex in the epithelial cells of the glomeruli and — most important for our present investigation — also in the endothelial cells of the *dilated lymph capillaries of the hydronephrotic kidney*. Germanin-protein granules — partly free and partly ingested by phagocytes — were moreover encountered in the lumen of lymphatic capillaries.

To preclude the possibility of dealing with artefacts, two kinds of *control experiments were performed. The first* was to examine the granules in polarized light: we found that they are not polarizing, a good indication of the fact that what we saw were not just crystallized dye particles. The second method was to examine animals that had received no germanin: after treating the kidney of these animals in the same way as those of the tests we encountered no granules in the lymphatic endothelium — another indication of the fact that the observed granules were not mere artefacts.

The above-described investigations justify the conclusion that normal lymphatic endothelium has the power to ingest protein molecules. Whether such phagocytosis plays a role in the removal of interstitital proteins in normal circumstances or must be regarded as an indepedent phenomenon has not yet been decided. Similarly, the question as to whether the behaviour of the germanin-protein complex

In the first series of experiments, 10 ml of physiological saline containing 1% Congo red and 1% inulin were administered by the subcutaneous route to one leg and the same solution but containing also *hyaluronidase* to the other.

As was to be expected, the lymph flowing from the extremity contained inulin in fairly high concentration: immediately after the administration, its concentration in the lymph was more than 50 per cent of the original concentration. This level gradually diminished during the period of observation.

The absorption of inulin through the lymphatics was not found to be increased by hyaluronidase. This is especially noteworthy if we remember that — as has been noted in the preceding chapter — hyaluronidase markedly promotes the absorption of inulin into the blood stream.

The red colour of the lymph pouring out from the open vessel showed that the Congo red had entered it in the space of a few seconds. In the first phase of lymph collection, i.e. within two and a half minutes after the injection, the concentration of the dye in the lymph reached as much as 33 per cent of the dye level in the injected fluid, to diminish at a rapid rate thereafter. Although hyaluronidase did exercize a promoting effect on lymphatic absorption, it was very weak and manifested itself in some cases only through a slowing down of the rate at which the initial level of the dye decreased after the first phase of lymph collection. The effect of the enzyme was statistically significant, nevertheless ($p = 0{,}5\%$).

In the experiments with protein, 10 ml of human serum was administered subcutaneously in the hind leg of dogs, and it was by means of precipitation test that we determined the presence of serum in the lymph drawn from the vessel running along the saphenous vein. Hyaluronidase was added to the serum injected into one of the legs.

These experiments showed that the entrance of protein into the lymph vessels was decisively facilitated by the action of hyaluronidase. While, in the control experiments, no serum was demonstrable in dilutions above 1 : 200 to 1 : 250, the precipitation titre of the lymph went up to 1 : 800 and 1 : 1000 when hyaluronidase had been added to the injected serum (Table 38).

TABLE 38

*Effect of hyaluronidase on protein absorption*

| Number | Control | | | | | Hyaluronidase 5 mg/ml | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 30 sec | 1 min. | 4 min | 7 min. | 10 min | 30 sec. | 1 min | 4 min | 7 min | 10 min |
| 1 | 0 | 1 50 | 1 : 100 | 1 · 125 | 1 : 150 | 1 · 150 | 1 . 150 | 1 750 | 1 . 700 | 1 : 800 |
| 2 | 0 | ± | 1 100 | 1 200 | 1 250 | 1 : 250 | 1 · 200 | 1 600 | 1 100 | 1 : 1000 |
| 3 | 0 | ± | 1 100 | 1 : 200 | 1 . 150 | 0 | 1 250 | 1 800 | 1 . 800 | 1 : 1000 |

is in every respect similar to that of free and undenatured plasma proteins is undecided.

What we may claim with assurance is that proteins can be stored in the endothelial cells of *lymphatic capillaries. This, presumably,* applies also to paraproteins. Our investigations furnished in any case an objective experimental proof of the correctness of Randerath's and *Fresen's findings.*

## EFFECT OF HYALURONIDASE ON THE PERMEABILITY OF LYMPHATIC CAPILLARIES

Whether a substance that has gained access to the interstitial tissue spasses into the lymphatics depends — as has repeatedly been pointed out — not solely on the permeability of the lymph-capillary walls but also on that of the interstitial tissues. So far, authors do not seem to have considered this aspect of the matter in full detail. We, therefore, deemed it imperative to examine the effect on absorption *into the lymph vessels of those factors which we knew to influence* spreading in the connective tissue.

Earlier experiments (Földi, Rusznyák and Szabó 1949c; Eöllős, Földi, Rusznyák and Szabó *1949)* had the object of studying the action of hyaluronidase on the penetration of colloidal and non-colloidal molecules as also of corpuscular elements (bacteria) into the lymphatics.

Absorption of subcutaneously introduced substances is known to depend on the size of their molecules. Barnes and Trueta (1941), for instance, claim that substances with a molecular weight over 20 000 are absorbed by the lymphatics from the subcutis as are inert corpuscular particles and bacteria, whereas substances with smaller molecules are absorbed by blood capillaries. These authors used snake venom and bacterial toxins in their experiments. Indian cobra poison with a molecular weight of about 2500 to 4000 was of the lowest mol. weight applied by them. In an attempt to find a substance of approximately the same mol. weight which would, at the same time, lend itself *suitably to chemical determination,* we chose inulin for our experiments: it has a mol. weight of about 5000 and can be determined with comparatively simple methods. Another of our test substances was *Congo red: its mol.* [illegible] (0 1 M) amounts, according to [illegible] ts, to some 9 000 to 10 000. [illegible] rotein (human serum) *and bacteria (an apathogenic strain of Bacillus anthracis*) into the lymphatics.

Our procedure *was to isolate those lymph ducts which run* along the Vena saphena in both hind legs of the dog and inject 10 ml of the fluid into the paw. Using a capillary pipette, we collected lymph from the lymphatic vessel at 1, 4 and 10 minutes after the injection and determined its inulin, dye, protein and bacterial content

the first place, since the comparatively wide pores constitute presumably *no barrier for smaller molecules*. The *experimental results of* Wasserman and Mayerson (1952a, b) allow namely the conclusion that not even the diffusion of relatively large protein molecules is essentially impeded by the walls of the lymph capillaries. In the experiments, leading to such conclusion, a correlation was established between the disappearance from the circulation of intravenously administered albumin-globulin and their return to the blood path via the thoracic duct.

The value of the average regression line for the amount of protein eliminated from the blood path and *returned to it by the thoracic duct* was 1·53. This means that 65·4 per cent of the proteins are led back to the circulation by the thoracic duct. It is of significance that the same value applies to both albumin and globulin which means that the lymph carries off the same percentage of both protein fractions. That the A/G-quotient of the lymph differs from that of the blood plasma is, thus, due to a difference in the rate at which these protein fractions *diffuse from the blood capillaries and not to that at which they enter the* lymphatics. However, we do not want to suggest that the entry of colloidal molecules into the lumen of lymph vessels is not restricted by the lymph capillary wall, i.e. that the latter need not be regarded as a semi-permeable membrane.

Let us refer in this connection to the findings of Hollander et al. (1956). They observed that the velocity at which subcutaneously *introduced colloidal particles were conveyed depended on their size* and solubility. While the half life of subcutaneously administered *crystalline* $I^{131}$ *is* (as measured at the site of *injection*) about an *hour*, that of $I^{131}$-labelled albumin is about a day. Any change in the solubility or the size of the protein considerably affects the rate of transport. A precipitation of the protein by heat at its isoelectric point raises its biological half life to 1·6 day. The half life of the protein precipitated by neutral salts is 1·8 day, and 2 days if precipitated by heavy salts. The absorption of the protein becomes still longer if oil or cholesterol is *added*.

Coming now back to the absorption-promoting effect of hyaluronidase: *in our publication of* 1949, *we interpreted this phenomenon by* the assumption (based on another alternative) that, by loosening the structure of the connective tissue, hyaluronidase facilitates the diffusion of larger elements and so promotes their penetration into the lymph vessels. This effect is of little significance for diffusible smaller molecules. It was for this reason that we recommended for medical practice the application of hyaluronidase for the purpose of accelerating the absorption of those large-molecular drugs which are mostly taken up and conveyed by lymph vessels. We have, moreover, found that hyaluronidase *promoted also the absorption of smaller molecules* (e. g. inulin) into the blood stream so that its application may be useful in *this respect as well*, although the promoting *effect* is less striking in

Experiments with anthrax bacilli yielded still more convincing results. The injected fluid contained 500 or 1000 million germs per ml; the highest number of germs found in the lymph *did not exceed* 1 million per ml if the injection had been administered without hyaluronidase and went up to nearly a hundredfold (30 to 100 million per ml) when the injected fluid contained enzyme.

Results are assembled in Table 39.

TABLE 39

*Effect of hyaluronidase on the passage of bacteria in the lymphatics*

| Number | Million germs/ml of lymph | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Control | | | | | | Hyaluronidase 5 mg/ml | | | | |
| | Administered | 30 sec | 1 min. | 4 min | 7 min. | 10 min | 30 sec. | 1 min. | 4 min. | 7 min. | 10 min. |
| 1 | 1000 | 0 | 0·8 | 1·0 | 1·0 | 1·0 | 15 | 15 | 75 | 100 | 75 |
| 2 | 500 | 0 | 0·2 | 0·5 | 0·5 | 0·5 | 10 | 20 | 20 | 30 | 30 |
| 3 | 500 | 0 | 0·3 | 0·3 | 0·4 | 0·4 | 10 | 15 | 25 | 30 | 25 |

We see that hyaluronidase had no effect on the inulin level of the lymph, increased the concentration of Congo red by not more than 10 to 20 per cent, but already that of serum to about the fivefold and increased the concentration of anthrax bacilli almost a hundredfold. It can undoubtedly be shown that the larger the size of the molecules or particles the more vigorously will their lymphatic absorption be enhanced by the enzyme.

We were the first to describe the effect of hyaluronidase on absorption through the lymphatics. The phenomenon has since been confirmed by several workers. It has been recommended that hyaluronidase be added to those dye solutions which it is customary to inject into the area of operation with a view to making lymphatic channels and regional lymph nodes visible in order to find carcinomatous metastases ("lymphangiography", Kinmonth 1952).

It should be noted that — according to Hollander et al. (1956) — substances possessing anti-hyaluronidase effect, e.g. hesperidin, do not effect the absorption of protein (albumin labelled with $I^{131}$) from the subcutis.

Several theories are possible to explain the reason why the action of hyaluronidase varies with the size of subcutaneously introduced particles. One of the conceivable possibilities would be that, by enlarging the pores in the lymph-capillary walls, the enzyme increases the permeability of lymphatic channels. This would facilitate the passage of larger particles into the lumen of the lymp capillaries in

In general, physiological saline containing 1% Congo red or trypan blue was diluted by dog *serum* at the rate of 1 : 1. This mixture was then injected into the one, and physiological saline containing 0·5% Congo red and *on serum* into the other leg of the animals. We had ascertained by means of ultrafiltration that Congo red, in a concentration of 0·5%, was quantitatively fixed by serum albumin.

Congo red dissolved in serum appeared in the lymph in an invariably higher concentration than Congo red dissolved in Ringer's solution, and the difference was significant throughout the experiments (Fig. 150). Having convinced ourselves that the values approached the normal distribution to a satisfactory extent, we performed the mathematical analysis by means of Student's test also in these cases.

Our following experiments had the object of seeing how the entry into the lymphatics of another dyestuff of large molecular size, namely trypan blue, would be influenced by protein. It was a surprise to find that — unlike Congo red — trypan blue did not respond to serum albumin. We then, subjected the dye-serum complex to ultrafiltration and found that — in concentrations used in *our* experiments — the dye was not quantitatively fixed by the protein and that most of it passed through the ultrafilter. It will be recalled that the diffusion of similar concentrations of trypan blue in gelatin [illegible] fected by protein. We, therefore. [illegible] difference that. [illegible] in the inject. [illegible] concentration of the [illegible] the presence of plasma protein (Fig. [illegible] and diffusion experiments proved that, if applied in the lower concentration, the dye was adsorbed by the diluted dog serum.

Patent blue was tested in the next experiments. This dye, considerably more diffusible than the other two, penetrated the lymphatics with equal ease whether serum albumin had been added to the injected fluid or not. Nor did the presence of protein affect the *in vitro* diffusion of those concentrations of the dye which were applied in our experiments because it was not quantitatively *fixed* by plasma protein.

The last dye to be tested was *basic neutral* red: protein was found to considerably increase its absorption by the lymphatics.

It is clear from all these experiments that the presence of protein vigorously promotes the entry of dyestuffs into the lymph vessels. Our previous experiments showed that protein had a promoting effect also on their spreading in the connective tissue. Are we to interpret this phenomenon in the sense that the passage of colloidal molecules chiefly depends on diffusion in the connective tissue?

In our preceding discussion of the effect produced by plasma proteins on the diffusion of dyes, we attributed their spreading effect in the first instance to the fact that the fixation of the dyes to the ground substance of connective tissues was considerably impeded by their absorption to the serum albumin and we further expressed our view that diffusion was also facilitated by the colloid-osmotic pressure

such cases. Numerous reports, published subsequently to our investigations, have confirmed the fact that hyaluronidase increases the absorption from the subcutis of colloidal and non-colloidal molecules. Banks et al. (1949) have, for instance, found that the absorption of plasma protein labelled with radioactive iodine was considerably facilitated by hyaluronidase, and the same observation was made by Simon and Narins (1949) in connection with Neo-Iopax (a radio-opaque substance), while Bauer and co-workers (1954) recommend the use of hyaluronidase to accelerate the absorption of subcutaneously administered dextran solutions in haemorrhagic shock.

## EFFECT OF COLLOIDS ON THE ABSORPTION THROUGH LYMPH CAPILLARIES

We have recently instituted investigations with a view to examining how passage into the lymphatics was affected by other factors which had been found to increase the spread of dyes in the skin, i.e. the diffusion in connective tissue. With this end in view, we elaborated a standard method.

[illegible]

eighth minute after the administration of the fluid. The subcutaneous introduction of the relatively large amount of fluid was followed by the spontaneous outflow of lymph sufficient to allow the determination of its dye level. To facilitate comparison, the dye concentration of the lymph was generally expressed as a percentage of the concentration in the introduced solution.

It is reasonable to ask why it was the dye concentration in the lymph and not the actual quantity of dye escaping with the lymph that we subjected to examination. No doubt, the latter method would have been better, had it not required the accurate measurement of the total amount of lymph pouring out from the extremity. Such measurement is, however, practically impossible. Number and position of the efferent lymph vessels in the hind leg are extremely variable. It is therefore impossible to find all lymphatics and collect the total amount of lymph flowing in them. We think it would lead to erroneous results if we used Drinker's method; he measures the amount of lymph flowing out of a single vessel and multiplies the figure so obtained by three, the assumption underlying this procedure being that the number of efferent lymphatics is invariably 3, and the amount of outpouring lymph always identical for all of them.

It was for this reason that we preferred the determination of dye concentration and not that of the absolute quantity of dye carried by the outflowing lymph.

Preliminary experiments proved that our method was practicable and that it gave reproducible results if adequate precautionary measures were observed, that is to say, the concentration of dye was approximately identical in the lymphatics of both legs if identical solutions of the same dyestuff were injected on both sides.

The next series of experiments was designed to ascertain how the penetration of dyes into the lymphatics would be affected if protein were added to the injected fluid.

of the proteins. The significance of the first factor for the absorption through lymphatics is borne out by the results of our experiments with polyvinyl pyrrolidone (Szabó and Magyar 1954, cit. Szabó 1954). It was demonstrated in these experiments that absorption through the lymph vessels was significantly increased by PVP (Fig. 150). In this respect our results are, thus, in agreement with those obtained from diffusion experiments.

It was further found (Szabó and Magyar 1954) that the spread of dyes was more increased by dextran of low than by that of higher molecular weight. The fact that dextran does not bind dyestuffs led us to the conclusion that colloid-osmotic pressure was an important factor in the rate of diffusion. We now made analogous experiments to study the entry of dextran with both low and high molecular weight into the lymphatics and to ascertain the influence of both fractions on the passage of dyes into the lymph vessels.

We found that subcutaneously introduced dextran of lower molecular weight appeared in the lymph vessels in a concentration which was 30 to 40 per cent higher than that of the similarly administered fraction of higher molecular weight. When Congo red is added to the injected dextran, a little more of the dye will gain access to the efferent lymph vessel if the dextran has a low molecular weight, although Congo red is not bound by dextran.

It can be seen that the results of these experiments support the theory that the passage of colloidal molecules largely depends on diffusion in the connective tissues. We must nevertheless admit that a closer analysis of our results and their comparison with those obtained from experiments concerning diffusion in connective tissues reveal certain contradictions.

That serum fails to promote the entry of 0·5% trypan blue and 0·5% patent blue into the lymph vessels is in our opinion due to the fact that, in this concentration, the major part of the dyes remains free and is not adsorbed to albumin. The diffusibility of stains was markedly enhanced by serum under similar conditions in the experiments concerning diffusion. Additional investigations revealed further differences between the two types of experiments.

## EFFECT OF METABOLIC POISONS ON ABSORPTION BY LYMPH CAPILLARIES

It has been noted that spreading in the connective tissues is very markedly diminished by various metabolic poisons, e.g. potassium cyanide, sodium fluoride, dinitrophenol etc. Therefore, we proceeded to study the effect of these agents on the absorption of dyes through the lymph vessels.

Our above-described standard method was used in these experiments also: Congo red or trypan blue dissolved in serum was injected into one leg of the test animals, and the same solution containing also

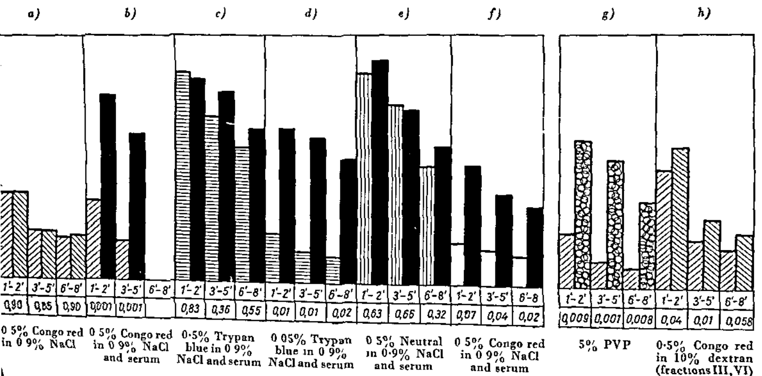



*Fig. 150.* Effect of colloids on the absorption through lymph capillaries

% = concentration of dyestuff in lymph in per cents of injected fluid; T = minutes after injection of dye solution; p = probability according to Student

the return of plasma proteins that had escaped into the interstitial tissues. It is known, however, that not only proteins but also all kinds of other "foreign" colloidal and corpuscular elements gain access to the lymph capillaries. It would therefore be reasonable to suppose that, aided by active cellular function, plasma proteins reach the lumen of lymph capillaries quicker than foreign colloidal molecules so that if Congo red is first attached to proteins and so administered, it, too, will profit by the active transport mechanism and get into the lymph vessels at a quicker rate. This concept seems to be confirmed by the results of our experiments with various other protein-bound dyes.

Zhdanov (1952), too, argues in favour of a "physiological" function of the endothelial cells. Of course, physiological function is not understood to mean some nebulous vitalistic process but that form of permeability which enables fluids or dissolved substances to pass against a gradient (of osmotic or hydrostatic pressure) into or through the living cell. This means work for the cells wich they perform with the aid of special enzyme systems and which depends on the liberation of metabolic energy. We have seen that, far from being impeded, the transport of the dye-protein complex was — in one case at least — apparently rather facilitated by ferment poison (cyanide). These observations seem, therefore, to be in contradiction to the concept that active processes are involved in the passage of dyes and proteins through the wall of lymph capillaries.

The fact, too, that not only proteins but also other colloids (PVP, dextran) promote absorption through lymph capillaries argues against the hypothesis which postulates an elective, active transportation of homologous protein molecules.

It has been noted that, while making our experiments with KCN, we were surprised to find that the penetration of Congo red seemed to be somewhat promoted by cyanide. If one considers this experimental result together with the well-known observation that lymphatics are postmortally easily filled with injected substances from the direction of the interstitial tissue, one is induced to consider the possibility that — far from actively absorbing the macromolecules from the interstitial space — the living endothelium of the lymphatic capillaries rather impedes their passage into the lumen of these vessels. Such considerations encouraged us to undertake investigations into the functioning of the lymph-capillary walls.

## ABSORPTION AFTER DEATH

Our standard method was employed also to investigate the lymphatic absorption in dogs 18 hours post mortem. Results were compared with those obtained from earlier experiments on living animals.

The values concerning penetration of Congo red dissolved in physiological saline showed no significant difference from those observed in living animals. A radical change was, however, observed when Congo

KCN in a concentration of $10^{-2}$—$10^{-3}$ M or dinitrophenol in a concentration of $10^{-4}$ M into the [illegible] not impede the absorption of e [illegible] and that, on the contrary, th [illegible] vessel was increased by an [illegible] period between 6′ and 8′; the increase was slight but, according to mathematical analysis, still significant. Cyanide in a concentration of $10^{-3}$ M did not affect lymphatic absorption. Nor did dinitrophenol in a concentration of $10^{-4}$ M produce effect on the absorption through lymphatics (Szabó 1954) although previous experiments had shown

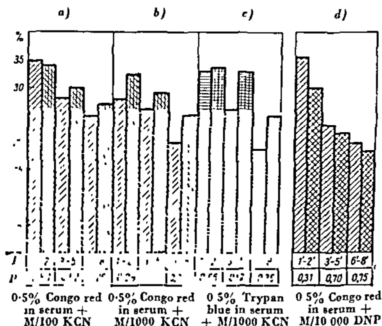



*Fig. 151.* Effect of metabolic poisons on the absorption trough lymph capillaries,

Left column control, right column substance tested Further keys as in Fig. 150

that it significantly diminished diffusion in the connective tissues and counteracted the spreading effect of hyaluronidase. These results tend to show that diffusion in the connective tissue, important as it is, does not constitute the sole factor in lymph formation. Besides diffusion in the connective tissue, the penetration of fluids and dissolved molecules into the lymph vessels depends also on other factors, in the first place on the permeability of the lymphatics.

Apart from the consideration that they indicate the absence of a parallelism between diffusion in the connective tissue and the process of absorption, we regard our experiments with enzyme poisons as significant also from another point of view. The most important physiological function of the lymphatic system obviously consists in

TABLE 41

*Appearance of patent blue (dissolved in physiol saline and serum) in the efferent lymphatic of living and dead animals*

| Nº | 1'—2' | | | | | | 3'—5' | | | | | | 6'—8' | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Living | | | Dead | | | Living | | | Dead | | | Living | | | Dead | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| | Phys NaCl | Serum | Diff | Phys NaCl | Serum | Diff | Phys NaCl | Serum | Diff | Phys NaCl | Serum | Diff | Phys NaCl | Serum | Diff | Phys NaCl | Serum | Diff. |
| 1 | 33 | 33 | 0 | 112 | 105 | —7 | 31 | 38 | —3 | 110 | 103 | —7 | 22 | 29 | +7 | 87 | 105 | +18 |
| 2 | 27 | 58 | +31 | 66 | 110 | +44 | 30 | 37 | +7 | 64 | 102 | +38 | 20 | 22 | +2 | 86 | 89 | +3 |
| 3 | 34 | 31 | —3 | 85 | 100 | +15 | 28 | 20 | —8 | 85 | 87 | +2 | 15 | 12 | —3 | 70 | 61 | —9 |
| 4 | 44 | 60 | +16 | 47 | 118 | +71 | 44 | 48 | +4 | 45 | 118 | +73 | 31 | 49 | +18 | 41 | 110 | +69 |
| 5 | 49 | 36 | —13 | 53 | 71 | +18 | 30 | 33 | +3 | 53 | 71 | +18 | 31 | 24 | —7 | 47 | 66 | +19 |
| 6 | 52 | 36 | —16 | 57 | 41 | —16 | 34 | 31 | —3 | 57 | 42 | —15 | 11 | 19 | +8 | 52 | 38 | —14 |
| 7 | 13 | 21 | +8 | 73 | 74 | +1 | 12 | 12 | 0 | 69 | 62 | —7 | 10 | 10 | 0 | 60 | 57 | —3 |
| 8 | 17 | 16 | —1 | 77 | 77 | 0 | 21 | 14 | —7 | 74 | 71 | —3 | 13 | 12 | —11 | 74 | 70 | —4 |
| Average. | 33·6 | 36·4 | +2·75 | 71·2 | 87·0 | 15·7 | 28·7 | 27·0 | —0·875 | 69·6 | 82·6 | +12·4 | 19·1 | 22·1 | +3·0 | 64·6 | 74·5 | +9·9 |

| | Living Phys. sal.-serum | Dead Phys sal.-serum | Diff | Living-dead Phys-sal | Living-dead Serum | Living Phys sal-serum | Dead Phys sal.-serum | Diff | Living-dead Phys sal | Living-dead Serum | Living Phys. sal serum | Dead Phys. sal.-serum | Diff | Living-dead Phys. sal | Living-dead Serum |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1—2 | 4—5 | 3—6 | 1—4 | 2—5 | 7—8 | 10—11 | 9—12 | 7—10 | 8—11 | 13—14 | 16—17 | 15—18 | 13—16 | 14—18 |
| t | 0·505 | 1·541 | 1·123 | 4·214 | 4·756 | 0·166 | 1·176 | 1·240 | 5·118 | 5·493 | 1·085 | 1·048 | 0·700 | 6·653 | 5·310 |
| p | 63% | 17% | 28% | ≪ 0·1% | ≪ 0·1% | 67% | 28% | 24% | ≪ 0·1% | ≪ 0·1% | 31% | 33% | 50% | ≪ 0·1% | ≪ 0·1% |

red dissolved in serum had been injected: while, during the first period of lymph collection, the concentration of the dye in the fluid leaking from the lymphatics of live animals amounted, on an average, to 30·5 per cent of the original (injected) concentration, this percentage rose to 54·4 per cent in the case of dead animals, a very significant difference indeed ($p = 0{\cdot}1\%$). The difference became still more conspicuous during the second period: 24 per cent in living animals against 50·4 per cent in dead ones.

In other words: the effect of serum raised concentration (expressed in per cent of the concentration of the injected dyestuff) in the first period by 17·5 (a 142/per cent increase) in the living and from 15·8 to 50·4 per cent (a 240 per cent increase) in the dead animals which proves that the entry of Congo red into the lumen of lymph capillaries is much more vigorously increased by serum proteins in the dead than in the living organism.

The abvious explanation for the observed differences between the absorption in living and dead animals seems to be that in the living organism the dye is diluted by the tissue fluid and capillary filtrate which is constantly formed. This assumption cannot, however,

TABLE 40

*Effect of protein on the appearance of subcutaneously injected Congo red in the efferent lymphatic of living and dead animals*

| N° | 1'–2' | | | | | | 3'–5' | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Living | | | Dead | | | Living | | | Dead | | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| | Phys NaCl | Serum | Diff | Phys NaCl | Serum | Diff | Phys NaCl | Serum | Diff | Phys. NaCl | Serum | Diff |
| 1 | 20 | 38 | 18 | 20 | 64 | 44 | 13 | 32 | 19 | 10 | 53 | 43 |
| 2 | 11 | 31 | 20 | 14 | 43 | 28 | 7 | 29 | 22 | 10 | 54 | 44 |
| 3 | 11 | 23 | 12 | 18 | 58 | 40 | 4 | 20 | 16 | 11 | 58 | 47 |
| 4 | 9 | 27 | 18 | 13 | 39 | 26 | 5 | 16 | 11 | 7 | 25 | 18 |
| 5 | 22 | 32 | 10 | 12 | 51 | 39 | 9 | 25 | 16 | 5 | 48 | 43 |
| 6 | 13 | 40 | 27 | 17 | 70 | 53 | 4 | 36 | 32 | 10 | 63 | 53 |
| 7 | 6 | 23 | 17 | 17 | 56 | 39 | 3 | 12 | 9 | 9 | 52 | 43 |
| 8 | 12 | 30 | 18 | — | — | — | 6 | 22 | 16 | — | — | — |
| Average: | 13 0 | 30·5 | 17 5 | 15 9 | 54 4 | 38 6 | 6 4 | 24 0 | 17 6 | 8 9 | 50 4 | 41 6 |
| | 1—4 | | 2—5 | | 3—6 | | 7—10 | | 8—11 | | 9—11 | |
| t | 1 248 | | 5 262 | | 6·266 | | 1·712 | | 5·008 | | 5 392 | |
| P | 23% | | $\leqslant$0 1% | | $\leqslant$0 1% | | 12% | | $\leqslant$0 1% | | $\leqslant$0 1% | |

TABLE 42

*Absorption of 0·5% Congo red dissolved in serum, in dogs 1 hour after death*

| | In living animals | | | One hour after death | | | Difference | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1'—2' | 3'—5' | 6'—8' | 1'—2' | 3'—5' | 6'—8' | 1'—2' | 3'—5' | 6'—8' |
| 1 | 41·0 | 38·0 | 24·0 | 43·0 | 52·0 | 49·0 | 2·0 | 14·0 | 25·0 |
| 2 | 32·0 | 34·0 | 20·0 | 33·0 | 38·0 | 31·0 | 1·0 | 4·0 | 11·0 |
| 3 | 37·6 | 29·0 | 24·0 | 53·4 | 46·4 | 38·0 | 15·8 | 17·4 | 14·0 |
| 4 | 33·4 | 35·8 | 32·0 | 38·0 | 41·0 | 40·0 | 4·6 | 5·2 | 8·0 |
| 5 | 41·4 | 29·2 | 24·0 | 59·0 | 51·0 | 45·0 | 17·6 | 21·8 | 21·0 |
| 6 | 39·2 | 24·8 | 22·0 | 44·4 | 48·4 | 48·4 | 5·2 | 23·6 | 26·4 |
| 7 | 35·2 | 29·2 | 30·4 | 32·4 | 24·4 | 24·4 | —2·8 | —5·2 | —6·0 |
| 8 | 38·4 | 22·8 | 19·6 | 55·6 | 55·6 | 44·4 | 17·4 | 32·8 | 24·8 |
| Average | 37·5 | 30·2 | 24·5 | 44·7 | 44·6 | 40·0 | 7·1 | 14·4 | 25·5 |
| | | | | | | t = | 2·65 | 3·29 | 3·95 |
| | | | | | | p = | 3·5% | 1·5% | 0·8% |

Increased rate of absorption cannot be explained solely by differences in the spreading of the stain and its fixation in the connective tissue. We base this opinion on the different behaviour of Congo red and patent blue. We have seen that Congo red, dissolved in physiological saline, is attached to the fibres of connective tissues which prevents its access to the lymphatics. It is for this reason that the penetration of Congo red, administered in physiological saline, shows no increase in the dead animal. Bound to protein, Congo red can diffuse freely so *that, after death, it is easier for it to pass into the lymph capillaries* that have become more permeable. Patent blue, as has been demonstrated in the foregoing chapter, is comparatively less attached to the ground substance of the connective tissue, the dye is diffusible, difussibility is not affected by the presence of protein, so that — after death when lymphatic permeability is higher — the dye (whether dissolved in water or serum) enters the lymphatics in a higher concentration than in the living animal.

## ABSORPTION THROUGH LYMPH CAPILLARIES IN TRAUMATIC SHOCK

Noteworthy in connection with the above considerations are Malek's investigations (1955, personal communication). Knowing that subcutaneously and intravenously administered substances were absorbed at a much slower rate by animals in shock (Beecher 1943; Altmeier et al. 1951; etc.), Malek tried, on the one hand, to find the

be correct if only for the reason that in the experim[illegible] dissolved in physiological saline no es[illegible] dye level in the lymph of living and dea[illegible]

Using our standard technique, we [illegible] with anoth[illegible] pate[illegible] [illegible] experiments that serum prod[illegible] no significant effect on the penetration into the lymphatics in such concentrations of this stain as were applied by us. In vitro experiments showed moreover that serum also failed to affect the diffusion of patent blue into gelatin, at least under the given conditions of concentration. Observations on dead animals were essentially similar to those made on living animals: addition of serum made no significant difference [illegible] lymph[illegible] [illegible] [illegible] percentage of concentration in the i[illegible] in the efferent lymph vessels of dead animals than in those of living ones, and this irrespective of whether the dye had been administered in physiological saline or dissolved in serum. Dye concentration in the efferent vessel was almost as high as that in the injected solution: it amounted on [illegible] differ[illegible] [illegible] the three lymph[illegible] irrespective whether the dye had been administered in physiological saline or serum.

To sum up our results: penetration of Congo red into the lymph vessels of dead animals was significantly increased only if the dye had been administered with serum, while serum protein failed to influence the entry of patent blue into the lymph vessel both in the living and dead organism. Yet, the concentration of patent blue in lymph collected from the efferent lymphatic was invariably higher in dead animals, and it made no difference in this respect whether the injected dye had been dissolved in physiological saline or serum.

It should be noted that all these experiments had a common methodological fault, viz. that each type experiment was performed on another animal. This may have impaired the practicability of the method and reduced the reliability of our results. Our investigations of postmortal resorption were, moreover, performed 18 hours after death which justifies the objection that all observed differences may have been simply due to a decomposition [illegible] and the capillary endothelium. Led by [illegible] in a new group of experiments, the abs[illegible] after death on one and the same animal; the second investigation was made within an hour after death.

Results yielded by these experiments showed that the absorption of Congo red, dissolved in serum, had undoubtedly become more intensive as early as an hour after death than that observed in the same animal while alive (Table 42).

TABLE 42

*Absorption of 0·5% Congo red dissolved in serum, in dogs 1 hour after death*

| | In living animals | | | One hour after death | | | Difference | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1'–2' | 3'–5' | 6'–8' | 1'–2' | 3'–5' | 6'–8' | 1'–2' | 3'–5' | 6'–8' |
| 1 | 41·0 | 38·0 | 24·0 | 43·0 | 52·0 | 49·0 | 2·0 | 14·0 | 25·0 |
| 2 | 32·0 | 34·0 | 20·0 | 33·0 | 38·0 | 31·0 | 1·0 | 4·0 | 11·0 |
| 3 | 37·6 | 29·0 | 24·0 | 53·4 | 46·4 | 38·0 | 15·8 | 17·4 | 14·0 |
| 4 | 33·4 | 35·8 | 32·0 | 38·0 | 41·0 | 40·0 | 4·6 | 5·2 | 8·0 |
| 5 | 41·4 | 29·2 | 24·0 | 59·0 | 51·0 | 45·0 | 17·6 | 21·8 | 21·0 |
| 6 | 39·2 | 24·8 | 22·0 | 44·4 | 48·4 | 48·4 | 5·2 | 23·6 | 26·4 |
| 7 | 35·2 | 29·2 | 30·4 | 32·4 | 24·4 | 24·4 | —2·8 | —5·2 | —6·0 |
| 8 | 38·4 | 22·8 | 19·6 | 55·6 | 55·6 | 41·4 | 17·4 | 32·8 | 21·8 |
| Average: | 37·5 | 30·2 | 24·5 | 44·7 | 44·6 | 40·0 | 7·1 | 14·4 | 25·5 |
| | | | | | | t = | 2·65 | 3·29 | 3·95 |
| | | | | | | p = | 3·5% | 1·5% | 0·8% |

Increased rate of absorption cannot be explained solely by differences in the spreading of the stain and its fixation in the connective tissue. We base this opinion on the different behaviour of Congo red and Patent blue. We have seen that Congo red, dissolved in physiological saline, is attached to the fibres of connective tissues which prevents its access to the lymphatics. It is for this reason that the penetration of Congo red, administered in physiological saline, shows no increase in the dead animal. Bound to protein, Congo red can diffuse freely so that, after death, it is easier for it to pass into the lymph capillaries that have become more permeable. Patent blue, as has been demonstrated in the foregoing chapter, is comparatively less attached to the ground substance of the connective tissue, the dye is diffusible, diffusibility is not affected by the presence of protein, so that — after death when lymphatic permeability is higher — the dye (whether dissolved in water or serum) enters the lymphatics in a higher concentration than in the living animal.

## ABSORPTION THROUGH LYMPH CAPILLARIES IN TRAUMATIC SHOCK

Noteworthy in connection with the above considerations are Malek's investigations (1955, personal communication). Knowing that subcutaneously and intravenously administered substances were absorbed at a much slower rate by animals in shock (Beecher 1913; Altmeier et al. 1951; etc.), Malek tried, on the one hand, to find the

cause of this phenomenon and attempted, on the other hand, to find a method to eliminate it because the phenomenon in question frequently caused considerable difficulties in shock therapy. The cause of diminished absorption is clear: shock provokes a serious disturbance in peripheral circulation; elimination of part of the capillaries from the circulation, reduction of the filtering and at the same time diffusing capillary area leads necessarily to a slower rate at which substances introduced into the circulation escape from the capillaries and also to a slower reabsorption of substances introduced into the interstitial tissue, their slower diffusion into the capillary lumina.

According to earlier — largely surgical — reports, lymphatics become dilated and the lymph flow is increased in traumatic shock. Laborit and Morand (1946) claim that, in contrast to crystalloid solutions, subcutaneously infused plasma is readily absorbed in shock. They recommend the observation of absorption for the purposes of diagnosis and prognosis. Malek, too, found that the absorption of camphor oil was significantly increased in shock, while — in accordance with earlier reports — that of morphine, a substance of low molecular weight, was much retarded. Aware of this difference, Malek went on to [illegible]

very slow, while Congo red and corpuscular elements were absorbed at an increased rate. Looked at from the point of view of the absorption of drugs as also from that of infective bacteria that have gained access to the organism through injury, this question is of great practical significance in shock.

Malek's investigations into the absorption of antibiotics, too, served practical purposes. The absorption of crystalline penicillin proceeds through the blood capillaries, while that of procaine penicillin fixed to a colloidal vehicle, through the lymphatics (Malek 1953). As is natural under normal conditions, the absorption of crystalline penicillin is quicker than that of procaine penicillin; the situation is reversed in shock, i.e. it is now procaine penicillin which is absorbed more readily, even quicker than in the normal, shock-free organism.

Though it is conceivable that — in traumatic shock — lymph capillaries become actually more permeable, Malek's results seem to show that the lymph flow, also, becomes increased in animals suffering from shock. Our experiments yielded, however, results which were in contradiction to Malek's findings: not only did lymph flow not increase but, on the contrary, rather decreased in shock. Considering [illegible]

an hour, an hour and two hours later. *Ischaemic shock was produced after a few days, and — after having removed the tourniquets — we once more examined the absorption of the dye-labelled dog serum.*

TABLE 43

*Appearance of s. c. administered Geigy blue in the blood of normal dogs and in ischaemic shock*

| | Normal dogs | | | | | Ischaemic shock | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 15 min | 30 min | 60 min. | 90 min. | 120 min | 15 min | 30 min | 60 min. | 90 min. | 120 min. |
| 1 | 0 | 0·12 | 0·27 | 0·39 | 0·54 | 0·08 | 0·15 | 0·15 | 0·23 | 0·30 |
| 2 | 0 | 0 | 0 | 0·23 | 0·30 | 0 | 0 | 0·08 | 0·15 | 0·15 |
| 3 | 0·09 | 0·09 | 0·34 | 0·66 | 0·54 | 0·11 | 0·11 | 0·22 | 0·22 | 0·22 |
| 4 | 0·03 | 0·03 | 0·05 | 0·09 | 0·23 | 0·09 | 0·09 | 0·09 | 0·30 | 0·33 |
| 5 | 0·03 | 0·09 | 0·21 | 0·30 | 0·42 | 0·03 | 0·03 | 0·03 | 0·03 | 0·03 |
| 6 | 0·03 | 0·03 | 0·05 | 0·05 | 0·09 | 0·05 | 0·05 | 0·05 | 0·11 | 0·18 |
| 7 | 0·18 | 0·30 | 0·45 | — | 0·57 | 0·05 | 0·05 | 0·18 | 0·24 | 0·24 |

It can be seen from Table 43 that, far from finding a more rapid absorption in dogs with ischaemic shock, a not insignificant retardation was observed. We are at present unable to explain the difference between our and Malek's results: it may perhaps be due to the fact that he experimented with rabbits and we with dogs. Malek also examined the amount of lymph, that is, he determined, the amount accumulated in the Cisterna chyli and found there more lymph in rabbits with traumatic shock than in normal individuals. We, on the other hand, encountered a diminished amount of lymph flow in the traumatized dog.

*Our experiments have convinced us that there is no justification for* the assumption that shock promotes absorption through lymph capillaries. Even if Malek's findings were to prove correct they could not be regarded as a definite proof of an increase in the permeability of lymph capillaries.

## RELATIONSHIPS BETWEEN LYMPH AND TISSUE FLUID

It would seem, therefore, that the entry of dissolved molecules into the lumen of lymph capillaries is somewhat *impeded in the living* organism by the lymph-capillary wall. This concept is, however, in contradiction to what we saw in the course of many other experiments: whenever the dilated lymphatics in oedematous areas were clearly discernible in histological sections, the fluid in them contained — as was shown by their staining — more protein than the oedema fluid in their immediate vicinity. *Figures 152 to 154 will* well illustrate the matter at issue. It can be seen that the lymph vessel which runs in the portal field of the liver is more intensively stained by haemotoxylin-eosin, *i. e.* has *a higher protein level, than the closely* adjacent oedema fluid ("Mall's periportal space") (Figs. 152, 153). An inspection of Fig.



*Fig. 152.* Lymphoedema in the liver. A lymph vessel, a branch of the portal vein and Mall's periportal space easily visible. Contents of the lymphatic show considerably darker staining than the periportal oedema, indicative of higher protein concentration in the lymph



*Fig. 153. Lymphoedematous cat liver Fluid in the peri*portal, endothelially-lined lymphatic capillary shows darker staining than that in dilated Mall's space

154, illustrating a case of human pyelonephritis, will show that the lymph vessel in the renal parenchyma is much darker than the surrounding oedema fluid.

These observations are extremely important since they may help to decide the controversy concerning the identity of lymph and interstitial fluid.

Drinker and Field (1933) expressed themselves decidedly in favour of the theory that lymph and tissue fluid are identical. They suggest that the capillary filtrate invariably contains proteins in different

concentrations, although these concentrations may be lower than those of the lymph and possibly lower than those of the tissue fluid. While water and salts are reabsorbed by the capillaries from the filtrate, proteins pass into the lymph capillaries in the same concentration as that in which they are encountered in the tissue fluid. Lymph originating from different areas may contain different amounts of protein: they depend on the intensity with which water in those areas is reabsorbed by the blood capillaries. Lymph — according to Drinker

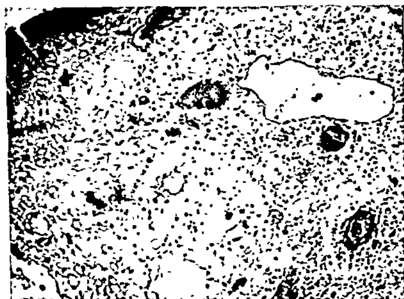


*Fig 154.* Lymph in the lymphatic darker than the surrounding oedemal fluid (pyelonephritis in man, H-E stain; photo by Gy. Romhányi)

— represents the cross section of tissue fluid contained in the area concerned.

Drinker continued to champion this theory (Drinker and Yoffey 1941) and adduced a number of arguments in its favour which may be summed up as follows:

Investigations made by Churchill, Nakazawa and Drinker (1927) showed the average protein content of frog lymph to be about 1 per cent (between 0·29 and 2·17%). Maurer (1938), inserting a fine needle into the musculature of the frog, determined the protein content of the so collected "extracellular fluid" which he found to be between 0·44 [illegible]

be found in the blood vessels. However, as has been noted (see chapter on the composition of lymph), lymph has a fairly high protein content. The protein concentration of the lymph in the thoracic duct amounts to about 75 per cent of the plasma concentration and even the leg lymph of dogs, which has the lowest protein level, contains on an average some 2 per cent, i.e. one third, of the protein content in the plasma, while, at the same time, there is nearly as much protein in the liver lymph as in the blood plasma. The extracellular interstitial space (after deducting the volume of circulating plasma from that of the sodium, sulphocyanide or inulin space) accounts for about 15 per cent of the body weight which corresponds to approximately 3 times the volume of plasma. Therefore, even if it is assumed that the whole mass of extravascular plasma protein is distributed over the interstitial space (though, in point of fact, the proteins of the lymph ought to be deducted from it), the protein concentration of the interstitial fluid cannot be more than some 30 per cent of the plasma concentration. We have seen that even the protein content of the leg lymph, which is especially poor in protein, has a higher value than this, while the value of the protein level in the mixed thoracic-duct lymph is about three times as high. It is thus quite evident that there is in any case less protein in the interstitial fluid than in the lymph and that, therefore, lymph and interstitial fluid must be two different substances.

Let us now examine the so-called barrier function of the lymphatic wall against the entry of colloids. Both physiology and pathology offer a fair number of instances in which the diffusion of some substance is hindered by a living membrane. We pointed out a few years ago (Földi, Rusznyák and Szabó 1918) that — apart from being actively involved in the processes of absorption, secretion and synthesis — renal tubular cells carry out the function of partly or completely preventing certain substances from being reabsorbed by the blood vessels. A lesion or destruction of the cells leads, on the other hand, to the passive rediffusion of these substances. A lesion of its cells causes the normal intestinal epithelium to lose its impermeability to sulphate ions: intact intestinal mucosa has, therefore, the function of impeding the entry of certain substances into the blood stream. Led by such analogies, we have recently come to the conclusion (Foldi, Rusznyák, Szabó and [illegible] e func- [illegible] mate- [illegible] have to [illegible] olutely necessary to suppose that the endothelium of lymph capillaries has the function of actively hindering diffusion, for the phenomenon in question may well be similar to that observed in blood capillaries.

Landis' investigations (1927/1928) made it clear that the normal permeability of blood capillaries invariably depends on normal metabolism. Metabolic poisons (mercury bichloride, cyanide, urethane,

etc.), as also anoxia of short duration, had the effect of giving rise to a considerable increase in capillary permeability under the conditions of Landis' experiments. A three-minute blockage of blood supply, for instance, was sufficient to induce increased permeability of the mesenteric capillaries which makes it obvious that the permeability of capillaries must become significantly higher after death. We do not propose to analyze now the mechanism responsible for increased permeability. Leaving open the question whether one is here dealing with a lesion of the endothelial cells by toxic substances or the grave disturbance of metabolism which leads to increased permeability, or whether all we are concerned with is just a change in the condition of the inter endothelial cement (e.g. enlargement of the pores), it seems to be obvious that the same noxious factors must produce similar damages in the structurally similar lymph-capillary walls. It is, therefore, more correct to define the observed phenomena by saying that both lymph capillaries and blood vessels become more permeable after death and that the endothelium does probably not actively impede lymphatic absorption in the living organism.

## MECHANISM OF ABSORPTION THROUGH THE LYMPH-CAPILLARY WALL

An additional object of our preceding arguments was to make it clear that the endothelium of lymph capillaries is the same kind of membrane as the endothelium of blood capillaries, although there exist certain — at least apparent — differences between the two. Blood-capillary walls are semipermeable membranes, almost complete-
[illegible] it know-
[illegible] passage
[illegible] to point
out, however, that no such fundamental difference between the two membranes exists in reality. Blood capillaries, also, allow the passage of protein to some extent; we have seen that protein escapes also from peripheral capillaries, and it is quite possible that capillary permeability is still higher in parenchymatous organs, presumably in the liver in particular. The entry of colloids into the lymphatics is, in the other hand, impeded to some degree also by the lymph-capillary walls (see preceding chapter). As regards the passage of corpuscular elements into the lymphatics, it should of course be noted that such *particles, e g. erythrocytes and leucocytes*, cannot — under physiological conditions — gain access to lymphatics unless they have first escaped from the blood capillaries, although this presumably presupposes a certain damage of the capillary walls. We saw, for instance, in the course of our experiments that lymph had become blood-stained under the effect of anoxia. However, lymphs shows traces of blood also by the action of agents which are not usually supposed to provoke capillary lesions. We observed, for example, that intravenous

administration of a mercurial diuretic (novurit) promptly caused the lymph to become blood-stained. It was, moreover, demonstrated by several authors that intravenously-introduced corpuscular elements (India ink, graphite, calcite, bacteria, filariae etc.) were rapidly eliminated from the blood (Drinker, Enders, Shaffer and Leigh 1953; Augustine and Drinker 1935; Landis 1934; Field and Drinker 1936; Baron and Chambers 1935/1936 and others).

As regards corpuscular elements, it must be admitted that neither their escape from the blood stream nor their entry into the lymph capillaries has, so far, been satisfactorily explained. Microscopically visible particulate matter cannot escape through the submicroscopic pores of the capillary wall, for — as has been noted — they are so small as to make even the passage of protein molecules (a thousand times less in size than corpuscular particles) rather difficult.

It is supposed by Jancsó (1941) that phagocytosis of corpuscular particles by the endothelial cells of the blood capillaries may play a certain role in this connection; histamine modifies the function of endothelial cells by causing them to bind and granularly store the circulating colloids and India ink particles, that is, to behave like cells of the RES. He observed this phenomenon in rats, guinea pigs, mice and cats which were first treated with histamine and then given India ink by the intravenous route. If, for example, histamine was rubbed *into the abdominal skin of rats, there appeared after the injection of* India ink a greyish black spot in the rubbed area, a phenomenon due to the fact that — activated — the endothelium of small vessels and capillaries of the subcutaneous connective tissue and the musculature *had phagocytosed the particles of the dye.*

Mention has already been made of Henry's investigations (1933) which led him to assume that India ink particles escaped through temporary apertures in the capillary wall; we have likewise referred to the experiments of Clark and Clark (1936/1937) according to whom oedema, by dilating the lymph vessels, gives rise to the formation of slits between the endothelial cells.

A further fundamenta [illegible]
laries seems to be that b [illegible]
"directed", i.e. *one-way,* [illegible]
*cannot be reabsorbed by them; once it has leaked out of the blood* capillary, protein has to be carried off by the lymph vessels. This difference also is, however, only an apparent one, it being obvious that it is not possible for the protein to pass back from a place of lower [illegible]

[illegible]

ally known, heterologous serum was mostly absorbed through the lymph capillaries in normal conditions — horse serum injected into the subcutis of dogs after plasmapheresis, i.e. animals in which hypoproteinaemia was induced, almost completely failed to appear in

the lymph but appeared promtly and in a relatively high titre in the blood. It follows that blood capillaries have no "one-way" permeability, at least not like that observable in frog skin.

Such evidence justifies the claim that there is no fundamental difference between the permeability of lymph and blood capillaries. Essentially, both kinds of capillary walls are semipermeable filtering membranes. If we consider moreover all the information furnished by recent investigations concerning the permeability of blood capillaries, it must seem very probable that also in the case of lymph capillaries, it is not through the endothelial cells but through the interendothelial cement, or rather through its pores, that water- and lipid-insoluble

the theory of Drinker (Drinker

Ve have seen that this author identifies interstitial fluid with lymph and does not regard the lymph-capillary wall as a semipermeable membrane which has the power to prevent the free diffusion of water and dissolved molecules into the lumen of the lymph vessels. Wasserman and Mayerson (1952c) found that their experimental results supported this theory. They observed that both the comparatively small albumin molecules and the comparatively large globulin molecules entered the lymph capillaries at the same rate. We do not think, however, that these findings justify conclusions concerning the permeability of the lymph-capillary wall. It is stated in the report of the authors that the same percentage of labelled albumin and globulin as has leaked out of the capillaries is returned by the thoracic duct. It is, however, obvious that the permeability of the blood-capillary is the principal restricting factor in the entry of proteins into the lymph. The proteins which escape from the capillaries and those which are returned by the lymphatics are, moreover, in equilibrium as has been recognized also by Wasserman and Mayerson. It is this state of equilibrium which is essentially reflected by the results of these authors so that conditions of concentration give no clue to the permeability of lymphatics.

Apart from this, the permeability of lymph capillaries may be greater than that of blood capillaries (in any case greater than that of the peripheral muscle capillaries we have taken as examples). The difference may simply be due to a difference in the submicroscopic structure of the cement of the lymph-capillary wall: the latter may

ies are united by abundant interstitial substance. Jancsó, too, admits that the endothelial cells of the lymph capillaries have a characteristic which differs somewhat from that of blood capillaries; their contour resembles the design of oak leaves, and the edge of the cells displays wide and deep dentations. These morphological differences are

summed up by Zhdanov (1952) as follows: the edge of lymphatic endothelial cells (in contradistinction to blood capillaries) is not smooth but — as is well-demonstrable by the silver impregnation of the intercellular substance — decidedly serrated. Lymph capillaries have no basement membrane, their wall consists of a single layer of endothelial cells (see in this connection Bartels 1909). It is therefore possible that the absence of a basement membrane and the greater width of the interendothelial cement account for the greater permeability of lymph capillaries.

Zhdanov further points to the close correlation that exists between the endothelial cells of lymph capillaries and the fibres of connective tissues, a correlation first demonstrated by Pullinger and Florey (1935). Zhdanov regards interendothelial cement and the ground substance of the connective tissue as identical substances, an identity that applies according to several authors also to the interendothelial cement of blood capillaries (see report of Chambers and Zweifach 1947). One of the chief arguments advanced in this connection was that hyaluronidase increased the permeability of both connective tissue and capillaries. Since, however, evidence is accumulating to show that the factors promoting dermal spreading are not the same as those which enhance capillary permeability, the argument in question can no longer be accepted. On this basis we feel justified in doubting the identity of the interendothelial cement in lymph capillaries with that of the ground substance of connective tissues. The observation that the same factors frequently influence the diffusion in connective tissues and the penetration into lymphatics not in a like but in an opposite sense seems to support our views. Let us refer to the effect [illegible] es but do not [illegible] the entry of colloidal molecules into the lymphatics. Again, while the spread of colloidal dyestuffs in the connective tissue decreases, the permeability of lymph vessels increases after death, etc. Such differences argue in any case against an identical composition of connective-tissue ground substance and interendothelial cement, although the possibility of such identity must not be disregarded altogether.

A close connection between lymphatic endothelium and connective-tissue fibres entails also another consequence, extremely important from the viewpoint of lymph circulation. As long ago as 1921 Clark and Clark demonstrated that with rising tissue pressure in oedema formation, lymph capillaries — unlike blood capillaries — become dilated instead of being compressed. This is due to loosening of the ground structure which causes the fibres of the connective tissue to move apart so that — being, as they are, attached to the walls of the lymph capillaries — they simply pull the walls apart and thus prevent a compression of the vessels.

The experimental results and morphological observations quoted in the foregoing do not, therefore, invalidate our theory that the wall

of lymph capillaries should be regarded as a membrane whose essential properties are not very different from those of the wall of blood capillaries. There are, as is only natural, certain differences between the respective functions of the two membranes. While filtration through the blood-capillary wall takes place from a fluid which is under high pressure and has a rapid flow, the conditions of pressure in which fluid is flowing from the interstitial space to the vascular lumen through the lymph-capillary wall are utterly different inasmuch as there is practically no gradient between extracapillary and intracapillary pressure and the stream in the vessel is comparatively sluggish.

Let us therefore examine the manner in which fluids, dissolved crystalloid and colloidal molecules pass through the membrane.

Lymphatic capillaries may be regarded as clefts in the connective tissue which, although lined by an endothelial layer, are — as has been noted — permeable to water and dissolved molecules. Fluid is, thus, *flowing into and filling the lymph capillaries. At rest there is,* at the periphery at least, no lymph flow in the lymph capillaries. When, however, a movement of the animals causes its muscles to contract, the thin-walled capillaries undergo compression, the fluid is pressed out of them and streams towards the small collecting lymphatics. Flow in these collecting channels is, however, always unidirectional since even the smallest postcapillary lymphatics contain valves which prevent a backflow of the lymph. When movement stops, e. g. when muscles become relaxed, it is from the interstitial space that fresh fluid flows into and refills the empty lymph capillaries (since lymph, once it has been pressed into the small lymphatics, cannot return to the capillaries).

This mechanism is in harmony with the experimental data we possess in regard to the formation of peripheral lymph. When at rest, there is no spontaneous flow of lymph from the peripheral limb lymphatics, a finding made by Genersich as long ago as 1871. If, however, the area in question is actively or passively moved or massaged, a considerable quantity of lymph will pour forth (Drinker and Yoffey 1941). That not merely the preformed lymph stagnating in the lymphatics, but also constantly reproduced fresh lymph is evacuated in this case is proved by the fact that the rate of lymph flow remains constant whatever the duration of the movement or massage.

Cannulating the efferent lymphatic of the hind leg of dogs, Haynes (1932a) observed lymph flow from the limb even in passive movement. What he saw was, therefore, not a phenomenon which might be conceived in connection with the experiments of Drinker and Field (1933), namely that active movement and muscular activity increase the blood supply of and the capillary pressure in the limb, and that increased filtration increased hereby promotes the production of lymph. It should be noted that — unlike Haynes — Drinker and Field studied lymph flow in the leg of unanaesthetized animals and found that lymph flow

was more vigorous when the dogs were walking or running, while hardly any lymph escaped from the cannulated vessel of quiescent animals.

A similar method was elaborated by McCarrell (1939a, b, 1939/40, 1940) for the collection of lymph from the cervical lymph trunk of anaesthetized dogs. After inserting a cannula into the cervical trunk, the head of the animals was passively moved in a steady and uniform manner by means of an ingenious device. Studying the rate of lymph flow under such experimental conditions, McCarrell found that, while there was hardly any flow from quiescent animals, lymph flow became very marked when movement began, and had diminished somewhat after half an hour. The amount of outpouring lymph per unit of time became constant after this and remained so for a long time. The initial rapid flow was obviously due to the fact that a considerable amount of lymph accumulated in the lymph capillaries and efferent lymphatics during quiescence, while the subsequent steady and uniform flow must have been that of newly produced lymph. With a view to inducing a passive movement of the legs of dogs and collecting the outpouring lymph we (Szabó 1954) elaborated a technique similar to that of Haynes (see Chapter V) and were able essentially to confirm the findings of earlier authors. These experiments have, thus, proved that, at the periphery, filtration exceeds fluid absorption even in quiescent animals, and that fluid is steadily accumulating in the interstitial space if only at a slow rate; true, the amount of this fluid is not very important, but is still sufficient to invalidate the assumption that, at rest, capillary filtration and absorption are in equilibrium. This is confirmed by certain generally known phenomena. It has been observed that even otherwise completely normal persons may develop oedema in the lower extremities after sitting long at the same spot (e.g. "deckchair disease" during long sea voyages) although, as a matter of fact, pressure in the dorsal veins of the leg is lower in sitting than in walking or at a time of maximum muscular contraction. The development of oedema in this case cannot, therefore, be solely due to venous congestion. It is also known that limbs forced to long inactivity frequently become swollen (e.g. immobilized by a plaster after fractures or the paretic extremities). Disturbance in all such cases is caused by the fact that the lymphatics fail to remove excess capillary filtrate: fluid enters the lymph capillaries, the lymphatics may even become dilated with developing oedema, but the lymph vessels do not carry off the fluid which, by its consequent accumulation, induces the formation of oedemas.

It may, of course, be argued that the rise of oedema in these cases can be due to the failure of the lymphatics to carry off protein which leads to an increased colloid-osmotic pressure of the interstitial fluid so that it is wrong to speak of a primary disturbance of water absorption. Such a polemic argument would in our view be useless since, as has been shown, the transportation of water is inseparable from that of protein. Capillary water filtration and absorption closely depend

on the protein permeability of the capillaries and the colloid-osmotic pressure of the intracapillary and extracapillary fluid. It would therefore be wrong to affirm that in the case of the above oedemas one was essentially dealing with a disturbance of protein transportation. We shall have occasion to show that the protein content of the oedema fluid is always high in disturbances of the lymph-circulation when oedema is developed. What this means is that the removal from the interstitial space of both water and protein is disturbed in such cases.

It is principally by way of diffusion that fluid passes from the interstitial tissue into the lymphatic capillaries. Also interstitial pressure — which is sometimes much in excess of lymph-capillary pressure — may, of course, play a certain part in this process so that the fluid is so to speak filtered into the lymph vessel; we know, however, from McMaster's results that this "filtration pressure" is exceedingly low in normal circumstances. It follows that diffusion is undoubtedly a much more important factor than filtration. Penetration into the lymphatics depends — as has been shown — not only on the rate at which fluids and dissolved molecules diffuse through the pores of the lymph-capillary wall but also on how they reach that wall, i. e. on the rate at which they spread in the interstitial tissue.

If the lymph-capillary wall is a semipermeable membrane provided with pores (of undetermined size), more diffusible molecules must be able to gain access to the lumen of lymph capillaries easier and in a higher concentration, while the entry of less diffusible colloidal molecules may be somewhat "impeded" by the capillary endothelium. How can we then explain our observation that protein concentration in the lymph is, nevertheless, usually higher than that of the interstitial fluid?

An obvious explanation seemed to be offered by the assumption that with lymphatics one was dealing with a "directed" permeability also: protein would, according to this hypothesis, pass into the lymph capillaries by means of active absorption without being able to get out of them. Data we have quoted in this work make it very improbable that active protein absorption (and, in general, absorption of colloids) occurs through lymph capillaries: the same arguments apply also against the hypothesis of a "one-way" permeability. We think, however, the fact itself that the permeability of lymph capillaries is not a "directed" one is important enough to justify our proving it by an account of the pertinent literature and of our own experimental results.

It was observed by McMaster and Parsons (1950) that, as was to be expected, [illegible]
readily in [illegible]
capillaries [illegible]
from the [illegible]
rapid, pontamin blue, less diffusible, at a slower rate.

Courtice and Steinbeck (1951) found that, after the intraperitoneal introduction of a mixture composed of protein and dye, the first to

become stained were the tissues adjacent to the efferent retrosternal lymphatics; they were followed later by the entire anterior mediastinum; after a certain time stained fluid accumulated in the thoracic cavity also.

Zhdanov (1952) observed that, if administered intralymphatically or even intraarterially, corpuscular elements (India ink, collargol) began to leak from the collecting lymphatics of the anterior mediastinum after a comparatively short time (1 to 2 hours) and, *once escaped, settled around the lymph vessels. Zhdanov attributed the escape of* the particles to phagocytosis by endothelial cells.

These experiments have thus proved that dyes which have gained access to the lymph capillaries are able to leave them again by way of rediffusion, further that corpuscular elements may be phagocytozed by the endothelial cells of medium-sized lymphatics and so eliminated from their lumen. We, however, desired to find out whether water and dissolved substances, too, were able to diffuse from the lumen of small and medium-sized lymphatics, and if so, whether the size of the pores in the vessel walls was such as to resist the leakage of *less diffusible large molecules.*

Experiments to this end were made with fluorescent dyes on cats. After having examined a number of such dyes we selected two: the first, a less diffusible stain, was known to be almost completely bound by *plasma proteins if applied in the given concentration;* the second, a more diffusible substance, was known to remain — partly at least — free, i. e. not to be fixed by proteins if used in the given concentration. Such experimental conditions enabled us to observe the behaviour of the dye-labelled protein as also that *of considerably smaller molecules in the lymphatics.*

Fluorescent thiazine red, injected into the intestinal wall of the cat, appeared in a short time in the efferent small lymphatics of the mesentery. The *vessels* showed a beautiful red fluorescence in UV-light. Fluorescent acridine yellow passed likewise into the lymphatics. We injected these dyes in a concentration of 50 mg%, *dissolved in a* 50-per cent dilution of homologous serum.

We found (Szabó 1954) that thiazine red had not leaked out of the lumen of the efferent lymphatic even after a longer period, whereas not more than 2 to 3 minutes after the administration of acridine *yellow the edges of the lymphatic became blurred* and the *surrounding* tissue displayed first pale and then vigorous yellow fluorescence. The injection of a mixture of both stains was readily followed by its appearance in the efferent lymph vessel with intensive reddish-brown fluorescence (Fig. 155). After the lapse of a few minutes, there appeared a *steadily increasing yellow fluorescence in the tissue* adjacent to the vessel, indicative of the fact that the more diffusible acridine yellow had leaked out of the lymphatic, whereas the less diffusible protein-bound thiazine red had remained in the vascular lumen (Fig. 156).

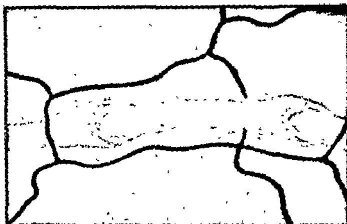


*Fig. 155.* Lymphatic, filled with dyestuff, in cat mesentery immediately after the injection into the intestinal wall of a mixture of thiazine red and acridine yellow dissolved in homologous serum

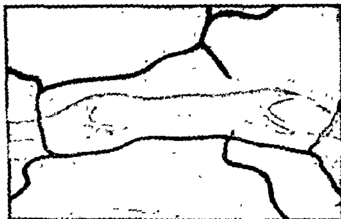


*Fig. 156* Lymphatic, represented in Fig. 155, a few minutes later. Acridine yellow passed through the vessel wall and stained the adjacent tissue

An *in vitro* experiment confirmed the fact that the two dyes are really different as regards diffusibility and their binding to protein.

In a concentration as used by us, the two dyes were dissolved in physiological saline and in serum, and then placed upon a column of 5% gelatin adjusted to pH 7.4. It took acridine yellow 48 hours to diffuse, with indistinct boundary line, into the gelatin to a depth of 15 to 20 mm, it making no difference whether the dye

had been dissolved in physiological saline or serum. Thiazine red penetrated during the same time to a depth of 10 mm if dissolved in saline, and to 6 mm if in serum.

[illegible], we found that rhodamine B and aesc[illegible]ymphatic very promptly, almost at [illegible]minutes; rose bengal, euchrysene and thioflavin needed 5 to 6 minutes for their escape. Of non-fluorescent dyes, brilliant cresyl blue (1% solution, in serum) appeared in the tissue surrounding the lymph vessel after 5 to 6 minutes, patent blue (in the same concentration) passed through the vessel wall in 6 to 8 minutes, while Geigy blue was still in the lumen after 15 minutes and also Congo red failed to escape from the lymphatic after a relatively long time of observation.

It is justifiable to infer from these results that the walls of the efferent lymph vessels, too, are permeable to dissolved molecules. They also permit the conclusion that permeability is somewhat limited: more diffusible molecules can more readily pass through the wall of the lymphatics into the interstitial tissue than less diffusible molecules.

Let us refer here to the earlier investigations of Landis (1927b) who studied the permeability of blood capillaries in the mesentery of frogs by means of various stains. He found that dyes introduced into the capillaries (trypan blue, trypan red, toluidine blue, brilliant red) penetrated through the capillary wall and that the rate at which they passed through it depended on the diffusibility of the dye, but also on intracapillary pressure. Toluidine blue, for instance, needs 5 minutes to emerge from the capillaries if the pressure in them is 7 to 8 cm water but appears in the extracapillary space within a few seconds if intracapillary pressure reaches 27 to 30 cm water. The most diffusible is trypan red which penetrates the capillary wall very quickly even at a pressure of 7 to 9 cm, while trypan blue needs a pressure of 12 to 13 cm. Congo red, the least diffusible of the dyes, does not emerge from the capillary unless pressure there reaches a value of 14 to 16, sometimes even 25 cm water.

We can see that the behaviour of blood capillaries and small efferent lymphatics is fairly similar. On the strength of our experimental results, as also on the analogy of experiments made on blood capillaries, we think we are now able to account for those contradictions in our results which emerged in the course of our investigations and, moreover, to make important statements concerning the origin and formation of lymph.

[illegible] intralymphcapillary pressure, [illegible]

mechanism, i.e. as a consequence of movement, external pressure or muscular contraction. Since intralymphatic and extralymphatic colloid osmotic pressure are more or less in equilibrium (we have seen that

intracapillary colloid osmotic pressure may even remain below interstitial pressure), the rise of the intravascular pressure may suffice to start a process of filtration through the permeable lymphatic wall. The permeability of the lymph-vessel walls is, however, not unrestricted. Less diffusible, i.e. colloidal and protein molecules can pass through the wall much less easily than do the more diffusible water and crystalloid molecules. *In consequence, protein concentration in the lymph* vessel will become higher than in the interstitial fluid, a phenomenon [illegible] investigations in which pressure in the large efferent lymphatics was determined by the insertion of a cannula into the terminal part of the vessel and its connection to a water monometer. Measured with this technique end pressure in the thoracic duct was generally found to have reached a height of 14 to 16 cm water (Weiss 1861; Lee 1923—24; Beck 1924, etc.). Under conditions which promote lymph flow, pressure may become still higher: Lee, for instance, measured pressures up to 35 cm in connection with forced inspiration. However, much lower values are obtained if not end pressure but lateral pressure is ditermined. Rouvière and Valette (1935) measured the lateral pressure in the thoracic duct in the following way: they isolated, and inserted the cannula into a small lateral branch of the thoracic duct. The pressure so determined was 6·4 cm water and, simultaneously, that in the internal jugular vein was 2·4 cm. Our own technique was to introduce a very fine needle into the thoracic duct (so fine as not to interfere essentially with the lymph flow) and connect the needle to a manometric system. With open thorax, the pressure in the duct generally did not exceed 4 to 5 cm water, but rose simultaneously with increasing venous pressure. McCarrell (1939b) measured lateral pressures of 2·8 to 3·2 cm in the cervical trunk, but the terminal pressure determined by means of a cannula inserted into the lymphatic was found to reach values up to 45 cm. Drinker and Field (1933) tied a T-shaped cannula into an efferent lymph vessel in the leg of dogs. While practically no lateral pressure was observable at rest, a vigorous passive movement of the leg brought the pressure up to 68 cm water. Ligation of the vessel drove the pressure up to not less than 100 cm.

High pressures were observed by Drinker and co-workers (1940) in the cardiac lymphatics (15·5 cm water), while Königes and Otto (1937) measured an average pressure of 33·3 cm water in the central lymph capillaries of the interstitial villi.

All these data seem to substantiate our theory concerning the mechanism of lymph formation. Let us point with especial emphasis to the observation made by Drinker and Field (1933) that even passive movement elicits a powerful rise of pressure in the leg lymphatics.

We think our hypothesis — which we have tried to support by a sufficient number of observations — explains those apparent contradictions which are encountered in the results of investigations into the mechanism of lymph circulation, and we hope it helps to elucidate certain hitherto unexplained phenomena.

Let us sum up the essential points: fluid that has passed from the interstitial tissue into the lymph capillaries and lymph vessels becomes concentrated in them as a consequence of a filtration process maintained by intravascular pressure. Since the passage of larger molecules is somewhat impeded by the wall of the lymphatics, protein concentration is lower in the escaped fluid so that the protein content of the fluid which remains in the lymphatic, i. e. of the lymph, will increase in proportion.

## DIFFUSION OF PROTEIN FROM LYMPHATICS INTO THE INTERSTITIAL SPACES

Lymphatics are, according to our concept, outlined in the foregoing paragraphs, permeable also to colloids, though less so than to water and crystalloids. It is known that lymph and chyle may gain access to the serous cavities and induce chylous ascites or hydrothorax. A damage of the thoracic duct caused by trauma or other reasons is demonstrable in many of such cases (Everhardt and Jacobs 1938), but it occurs not infrequently that chyle passes into the abdominal or thoracic and even into the pericardial cavity when the lymph-vessel wall shows no lesion, the lymphatics reveal no rupture but are blocked by tumorous compression or some other obstacle. It was supposed by *Hirschler and Buday (1889) that, in chylous ascites,* the fluid leaked through the wall of the dilated lymph vessels. Laplane, Lhermitte and Billard (1949), too, considered the possibility of diffusion through the wall of the lymphatics.

When lymph containing highly concentrated protein (e.g. lymph originating from a focus of inflammation) passes through an area of comparatively low protein concentration, protein may diffuse from the lymphatics. This hitherto insufficiently considered phenomenon may play a role in the mechanism of numerous pathological processes. We would refer in this connection to the report of Yessipova (1952) who studied the pathogenesis of pneumoscleroses which appear in so-called chronic aspecific pneumonias. She examined a total of 75 cases and frequently found in their history symptoms of relapsing pneumonias and chronic bronchitis. Post mortem more or less advanced pneumosclerosis was encountered in every case to which, in the majority of the cases, bronchiectasis, chronic relapsing pneumonic foci and often also emphysema were added. Significant from our point of view is the fact that sclerosis was mostly found to have developed in the vicinity of important collecting lymphatics. Of interest is the observation made by Erdélyi (1953) in our Institute that the pulmo-

nary lymph-vessel walls of enamellers and enamel atomizers who suffered from incipient silicosis were surrounded by a cicatricial coating.

Yessipova regards most of these pulmonary processes as acellular scleroses (fibroses) in which the fibrous elements develop directly, through accumulation and physico-chemical transformation of the interstitital substance, without a preceding multiplication of cellular elements. Acellular fibrosis arises always along or around lymphatics, and Yessipova assumes that the protein necessary for the construction of the interstitital substance is supplied by the lymph, large amounts of which pour forth from the inflammatory area. This lymph is known to contain much more protein than normal lymph. Lymph vessels filled with fluid of high protein content are clearly visible in the surroundings of the inflamed pulmonary foci. It is along these vessels that the transformation and accumulation of collagenous and argyrophile fibres begin.

Whenever fibrosis is found to develop in the surroundings of an inflammation or a necrotic disintegration of tissues, special attention should in our view be paid to the lymphatics of the area involved. Blockage of the lymphatics and consequent lymphatic congestion, i. e. accumulation of a fluid of high protein concentration in the interstitial space, is — as we shall have occasion to discuss in detail — an undoubtedly important factor in the genesis of fibrosis. There exists, as was shown, also another mechanism through which it is possible for lymphatics to promote proliferation of the connective tissue and develop cicatrization, viz. by way of a leakage of protein from the lymph vessels and an organization of the escaped protein. This process begins — characteristically — around the lymphatics inasmuch as organized protein, in the form of a cicatricial coat, surrounds the efferent lymph vessels of the inflammatory or necrotic foci (perilymphatic fibrosis). This is, of course, neither the only nor the decisive mechanism by which lymphatics contribute to the development of fibroses. Whatever the mechanism by which fluid, rich in protein, gains access to and remains in the connective tissue, it is always possible that it become organized there and so give rise to fibrosis.

On the other hand, every chronic insufficiency of the lymph circulation leads to the development of an interstitial oedema rich in protein which may then become organized and transformed into connective tissue.

## CONSEQUENCES OF CHRONIC LYMPH CONGESTION: FIBROSIS

Protein that has passed into the connective tissue does not remain there unaltered. Some authors hold that the proteins of the exudate are promptly precipitated, giving rise to the formation of a fibrinous network. Zimmermann and Takáts (1931) observed actually the de-

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in the vicinity of important collecting lymphatics. Of interest is the observation made by Erdélyi (1953) in our Institute that the pulmo-

because similar pictures had been observed also in apparently normal human thyroids (Földi, Jellinek and Szabó 1955a, b).

All these experiments seem to prove that after some time a proliferation of connective-tissue elements commences in the protein-rich fluid, irrespective of whether it is at the periphery (elephantiasis) in the kidney, the liver, or in another organ that the lymphatic stasis takes place.

In connection with these investigations it is reasonable to ask how the insufficiency of lymph circulation arises and what type of

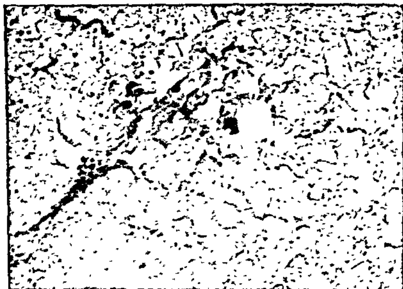


*Fig. 157.* Cat liver after 2 months of lymphatic obstruction Red-stained collagenous fibres in the Disse's spaces (Van Gieson's stain)

lymph-circulatory insufficiency one is dealing with in these cases. It must be borne in mind that whenever protein-rich exudate remains in the interstitital space for a longer period during which there appear later collagenous connective-tissue fibres, the inference is justified that one is dealing with some disturbance in the transportation of the exuded protein, i.e. that something is wrong with lymph circulation. For instance, chronic allyl formate poisoning was observed by Eppinger (1949) to be likewise associated with a multiplication of connective tissue and a fibrosis of the liver substance. He found that the toxic agent induced increased capillary permeability and the appearance of protein-rich oedema in the interstitial space. One has presumably to do with a dynamic insufficiency of lymph circulation in

velopment of such a fibrinous network in cold injuries: the lymphatics were obstructed by the fibrinous exudate so that they were unable to transport the escaped protein from the tissues. Fibroblasts are supposed to wander to the lymphoedematous area so formed leading to a thickening of the subcutaneous connective tissue, a multiplication of collagenous and elastic fibres, briefly, to the classic picture of chronic lymphoedemas.

It has long been known in human pathology that in chronic lymphoedema fibres of connective tissue appear in the originally protein-rich exudate: in elephantiasis, the most pronounced form of lymphoedema, for example, there arises a thick, massive and taut connective tissue, the skin is swollen and rigid so that it can no longer be lifted up in folds. The involved area is strongly swollen and sometimes deformed; the tumescence is hard, massive and does not retain the impression of the fingers which indicates the fact that there is no or hardly any oedema fluid in it, its place being occupied *by fibrous connective tissue.*

It was for a long time impossible to reproduce this chronic indurated form of the lymphoedema in experiments. Even the blockage of the total lymph circulation of a definite area provokes mostly a temporary lymphoedema only: the lymph vessels will either regenerate or form collaterals so that the oedema disappears in a few days or a week. After cutting through all soft tissues — save the vessels and nerves — in the hind legs of dogs, Reichert (1926) carefully sewed up the muscles [illegible] the deve[illegible] few days and disappeared very soon. Drinker, Field and Homans (1934) finally succeeded in provoking a state in dogs that was similar to elephantiasis in humans: they repeatedly ligated the lymphatics of the hind legs and injected thrombogenic substances into the closed vessels.

Natucci and Zaccarini (1949), after tying off the efferent renal lymphatics of dogs, observed after 40 to 50 days the development of collagenous fibres in the interstitital tissue.

In our own experiments, chronic lymphoedema was induced in the liver and the thyroid gland.

We ligated the efferent lymphatics in the liver, the animals were sacrificed after about 2 months and the liver were examined microscopically. Fig. 157 shows collagenous fibres stained bright red by Van Gieson's method in those areas of the cat liver which correspond to Disse's spaces.

Chronic lymph stasis in the thyroid of the dog was induced in an essentially similar manner: we ligated the principal efferent lymphatics of the head and neck, i.e. the two cervical trunks; the animals were killed 6 to 8 weeks later and the thyroids were histologically examined. Our pictures revealed fibrosis in the lymphatics as a consequence of chronic lymph congestion. They seem to us noteworthy

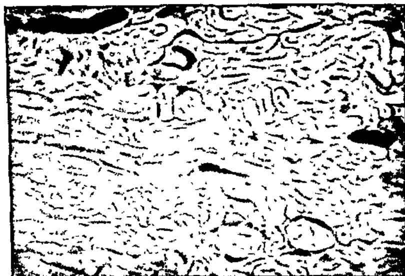


*Fig. 158.* Microphotogram of normal dog kidney (Haitinger's stain) in fluorescent light

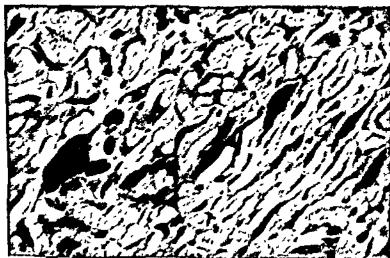


*Fig. 159* Microphotogram of dog kidney in fluorescent light (3 days of lymphatic obstruction)

this case: the lymphatics are unable to carry off the entire amount of protein and all the fluid that have accumulated in the interstitial space.

The transformation of protein-rich exudate into connective tissue and especially the nature of the mechanism by which the collagenous fibres of the connective tissue, are formed has been a battleground of conflicting theories for many a decade.

Investigations into the formation of embryonic connective tissue led Hueck (1920), for example, to the conclusion that collagenous fibres owed their origin to a condensation [illegible] blasts [illegible] lat [illegible] the fibrous elements of the [illegible] tissue arise.

Hueck's theory, adopted also by Standenath (1928), postulates a strictly cellular origin of the connective tissue fibres, i.e. that they arise from the cells themselves, while it was suggested by Ebner as long ago as 1896 that fibres originate not directly from cells, although from substances produced by the cells. Merkel (1909), on the other hand, advanced the theory that fibres might be formed without a participation of the cells since they arise from the intercellular substance. This view was shared by Russakoff (1909), Dubreuil (1913) and Hertzler (1915).

Baitsell (1916), as also Baitsell and [illegible] fibres probably arise from fibrin of pl[illegible] too, advanced a similar theory by sug[illegible] genous fibres, subsequently transformed by cellular ferments, arose from the coagulated fibrin. Eppinger (1949) explains fibrosis, observed in the liver in cases of allyl formate poisoning, by an enzymic transformation of plasma proteins.

We cannot see the probability of this concept. True, using Haitinger's fluorochrome technique, Eppinger succeeded in demonstrating the inhibition of the liver substance by the plasma proteins, while — after the ligation of the hepatic lymph vessels and with the use of a similar technique — we obtained pictures that were perfectly similar to those of Eppinger (Fig. 158). We also observed that a ligature of the renal lymphatics was soon followed by the appearance of protein in the interstices of the kidney which subsequently completely imbibed the renal substance, so that the picture of the kidney displayed a reddish or orange-yellow fluorescence in cases of lymphatic congestion, in contradistinction to the bright green fluorescence of normal kidneys (Fig. 159). Quite apart from the fact that we are not convinced of the correctness of Eppinger's assumption that Haitinger's staining is more or less specific for plasma proteins, we must point out that the theory according to which the presence of plasma protein is indispensable to the development of fibrosis cannot be regarded as fully proved. We have had occasion to observe a similar multiplication of collagenous fibres in the thyroid gland either as a consequence

this substance is produced by mast cells (Holmgren and Wilander 1937; Jorpes 1946). As had been found by Ehrlich, numerous mast cells are encountered in lymphoedematous areas. It was, accordingly, suggested by Ehrlich and his associates (1949) that there was a high concentration of heparin in the fluid of lymphoedematous areas. In a case of human elephantiasis it was possible to extract a consider- [illegible] safe to assume that heparin is secreted by the mast cells and that it accumulates in the area of elephantiasis.

We have had occasion to observe in the course of our investigations that the fluid contained in the Disse's spaces of lymphoedematous livers sometimes gives a metachromatic reaction with toluidine blue. It would be, of course, wrong to regard this stain as specific for heparin since other polysaccharide esters, hyaluronic acid for example, also show metachromasia (Pearse 1954). We have undertaken no experiments with a view to a precise identification of the metachromatic substance, although we are convinced that such experiments would be feasible and might contribute to the solution of the problems at issue.

Heparin is known to be a polymer consisting of glucuronic-acid and aminosugar (glucosamine) molecules (Jorpes 1946). Structurally, heparin is closely similar to hyaluronic acid, the only difference between the two being that, while aminosugar is acetylated in hyaluronic acid, a sulphate group appears attached to it in heparin. It was this consideration that led Asboe-Hansen (1951) to the conclusion that heparin or a heparinlike precursor of hyaluronic acid was secreted into the connective tissues by mast cells, to be acetylated and transformed there into hyaluronic acid. While the process under consideration is, in all probability, not quite as simple as this, a vast number of reports argue in favour of the supposition that mast cells play a significant part in the secretion of connective-tissue ground substance, particularly in that of hyaluronic acid (see in this connection Staemmler 1923 and, recently, Riley 1954). According to this theory, the process leading to a new growth of connective tissue, i.e. fibroplasia, when new collagenous fibres appear in the tissues, would take the following course: some precursor is first secreted by the heparinocytes and then transformed into hyaluronic acid; this is later followed by a condensation of the ground substance of the connective tissue, and it is from the condensed ground substance that collagenous and reticular fibres arise.

Notwithstanding the attractiveness of this theory, and the fact that there are many indications of a process of the described nature taking place in lymphoedema, we do not feel either justified or called upon to take a definite attitude as to its correctness.

of the ligation of the lymphatics or in connection with phenomena of senile retrogression. In this case the lymph and the fluid, extravasated from the lymphatics to the interstitial space, contain, however, not merely plasma proteins but also colloid originating in the acini.

Alfeiew (1926) and Maximov (1927) hold that although connective-tissue fibres arise independently of the cells they do not owe their origin to the exuded plasma proteins, the fibrin. Huzella (1929), experimenting with tissue cultures, made the same observation as Maximov, namely, that the origin of the fibrous network was extracellular. Similar results were obtained from the investigations of Bofill-Deulofeu (1932), Olivo (1933) and others.

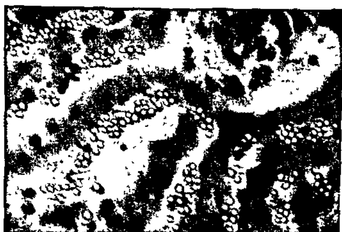


*Fig. 160.* Picture of lymphoedematous dog liver. Fluid in the Disse-spaces metachromatically stained with toluidine blue

All these reports seem to show that the fibres of connective tissues may in fact arise independently of cells but by no means prove that the fibres originate from blood proteins and from fibrin in particular. It is quite possible that they are formed from the ground substance of the connective tissue.

Of significance in this connection are investigations concerning the function of the so-called mast cells. Ehrlich (1879) was struck by the great number of what he described as mast cells in areas where lymphatics were obstructed and consecutive stasis developed. Many granules giving a metachromatic reaction with toluidine blue are contained in the mast cells. The finding of Jorpes that heparin stains metachromatic with toluidine blue and the observation that there exists a marked correlation between the heparin content of tissues and the number of demonstrable mast cells have given rise to the assumption that the metachromatic granules contain heparin and that

Although exudate, rich in protein, was contained in the interstitial space, no dilated lymph vessels to indicate increased lymphatic transportation were observable.

As in the cases of Rényi-Vámos and Róna, a mechanical or dynamical lymph-circulatory insufficiency seems to be improbable. It is much more likely that the failure of the interstitial oedema fluid to gain access to the lymph vessels is attributable to insufficient absorption by the lymph vascular system.

That interstitial fluid or protein fails to pass through the wall of the lymph capillaries and so get into the lumen of the vessels may, in principle, be due to various reasons. One of them could be given by the physico-chemical properties of the interstitial protein which prevent its passage into the lumen of lymph capillaries: this might be expected if the protein in the interstitial space differed from normal plasma proteins. It is in fact assumed by Randerath (1953) that, in cases of intercapillary glomerulosclerosis, one is dealing with paraproteinaemia or paraproteinosis (deposit of paraproteins in the tissues). How can paraproteinaemia induce an insufficiency of lymph circulation or, in other words, why are paraproteins not absorbed? We propose to discuss this question in connection with paraproteinoses where the presence of paraproteins has been proved beyond doubt. In this respect, we are thinking of multiple myeloma and the amyloidosis in the first instance.

In multiple myeloma, the protein produced in the malignantly proliferating plasma cells — a protein with properties different from those of normal plasma proteins — is filtered in the glomeruli and, as is proved by the hyaline droplets and crystals visible in the tubular cells, absorbed by the tubules. However, paraproteins are deposited in considerable quantities in the interstices of the kidney also. Protein deposited in the interstitial space may then lead secondarily to a proliferation of connective tissue. Accumulation of paraproteins in the interstitial space occurs in rare cases also in other organs (e.g. in the liver or spleen). It is in connection with such cases that Apitz (1940) uses the term "para-amyloidosis", assuming that proteins accumulating in the interstitial space and excreted by the kidney are different from proteins circulating in the blood path. Protein accumulates in the interstitial space of the kidney and elsewhere in the shape of crystals or protein coacervates which are called "structured" protein precipitate by Apitz. It is, therefore, presumable that protein — whether absorbed from the tubules or coming from the capillaries — is precipitated in the interstitial space in a crystalline or some other form and is thus prevented from entering the lymphatics. This assumption seems to be substantiated by the observations of Brass (1943, cit. Randerath 1953) and those of Wintrobe and Buell (1933) who found that, in cases of plasmacytoma, paraproteins circulating in the blood vessels or contained in freshly drawn blood may precipitate in the form of variously sized hyaline drops.

We think we can content ourselves with pointing to the consequences caused by chronic lymphoedemas, and the phenomena elicited by the accumulation of protein-rich exudate in the connective tissue, and that it suffices if we outline the mechanisms which can be supposed to be involved in the development of such conditions.

## INSUFFICIENCY OF ABSORPTION BY LYMPHATIC CAPILLARIES

When discussing the consequences of the presence of residual protein in the interstitital space we mentioned that one of the preliminary conditions was the failure of lymphatics to remove the remaining fluid, that is, an insufficiency of lymph circulation. We have dealt in the foregoing with the mechanical and dynamical insufficiency of lymph circulation, outlined the conditions in which such forms of insufficiency were developed and described their characteristic symptoms. However, the development of symptoms indicative of lymphoedema are sometimes observable in cases where neither a mechanical nor a dynamical lymph-circulatory insufficiency is demonstrable.

Renal parenchyma is known to be oedematous and the oedema to contain abundant protein in acute and subacute glomerulonephritis (Eppinger 1949). Examining such kidneys, Rényi-Vámos and Róna (1954) found that, in cases with a comparatively long course, the interstitital tissue contained collagenous fibres which gave a blue reaction with Mallory's stain. The picture of subacute cases was dominated by a proliferation of connective tissue and though the symptoms of chronic oedema were unmistakable, no (or hardly any) lymphatics were encountered in the substance of the kidney. The presence of oedema was obviously due to insufficient lymphatic drainage as no oedema could have developed if renal lymph circulation had remained normal. It is clear that lymph-circulatory insufficiency in the given case was not of a dynamic nature because a strong dilatation of the lymphatics ought to have been observable if lymphatic transportation *had reached its maximum and still remained inadequate.* Let us add that also a mechanical (organic or spastic) blockage of the efferent lymph vessels seems to be out of the question since a blockage of this nature would surely have entailed a dilatation of the small lymphatic capillaries at least.

We (Földi, Róna, Rusznyák and Szabó 1954a, b) examined the kidney of patients suffering from subacute glomerulosclerosis. What we saw was in essential agreement with what Rényi-Vámos and Róna had described in connection with acute and subacute glomerulonephritis (see Chapter on pathology of renal lymph circulation).

In cases of recent intercapillary glomerulosclerosis induced in these experiments, but also in cases where glomerulosclerosis had not — or not yet — developed, only interstitial renal oedema was encountered.

at those points of the human thyroid where symptoms of hyaline degeneration, fibrosis and — in general — chronic lymphatic congestion were visible in the glandular substance. This phenomenon is similar to that encountered in lobar pneumonia where, as is confirmed by pathological-anatomical observations, the pulmonary lymphatics are invariably involved in the pathological process. Not only is fibrinous exudate, similar to that which fills the alveoli, demonstrable in the lymph vessels but the endothelial cells of the pulmonary lymphatics, too, are swollen, even desquamated.

We must admit the possibility that such injured and diseased lymphatics become less permeable, although both experimental and pathological-anatomical results seem to point rather in the opposite direction. McMaster's (1942) experiments prove that the permeability of lymph capillaries in damaged skin is rather increased. This makes us incline to the view that the main factor responsible for the failure of the lymphatics to absorb proteins in both paraproteinosis and inflammations is not so much an injury of the capillary endothelium as the precipitation, crystallization of the proteins and their fixation to the structural elements of the connective tissue.

It must be remembered that, according to Menkin (1940b), lymphatics contain fibrinous thrombi in inflammatory processes. We observed in our own experiments that, after the ligation of the lymph vessels in experimental lymphoedemas, fibres of connective tissue began to appear also within the lymphatics, a phenomenon indicative of a transformation of the proteins into connective tissue. This seems to prove that the same process which occurs in the protein accumulated in the interstitial space, that is extravascularly, spreads over to that protein which has penetrated into the lymphatics. We are, thus, not quite in agreement with Menkin in this respect: he regards the formation of intralymphatic thrombi as one of the principal factors of fixation in inflammatory processes. We think alterations occurring intralymphatically are probably of a secondary character only and form but a part of general processes in the interstitial space. Thrombosis or a cicatricial obstruction of the lymphatics may of course contribute subsequently to the maintenance of disturbances in the transportation of proteins and fluids, but the primary cause of such disturbances will more probably be found in the interstitial tissue.

So far, we have confined ourselves to discussing the factors that may lead to disturbed absorption by the lymph capillaries but have not yet answered the question as to why the transportation of fluids and proteins was disturbed in our concrete examples, i.e. in glomerulonephritis and intercapillary glomerulosclerosis. We are, in our opinion, dealing also in these cases with an essentially complex process, similar to that described in connection with the thyroid. It does not seem probable that in acute diffuse glomerulonephritis paraproteins escape into the interstitial tissue to be crystallized or precipitated there, and this is the less so as Eppinger, using Haitinger's procedure

Similar phenomena occur in amyloidosis. Paraproteins circulating in the plasma escape into the interstices of the kidney, liver and spleen, but also into those of the muscles, e.g. the cardiac muscle. Amyloid was supposed by Randerath to be present in the interstitial space in an amorphous form but Romhányi (1949) succeeded in demonstrating that amyloid, precipitated in the renal medulla and the interstices of the cardiac muscle, assumed a crystalline structure. Protein precipitated and trapped in the interstitial space, may — as it does in other similar cases — lead to a proliferation of connective tissue and induce, for instance, amyloid degeneration of the kidney.

The question arises here as to why paraproteins are unable to gain access to the lumen of lymph capillaries: is such inability due to their physico-chemical properties or to other reasons (e.g. because lymph capillaries do not actively absorb paraproteins), or is it simply due to the fact that they become crystalline, i.e. fixed in the interstices and so unable to reach the lymphatics? We are inclined to the latter view which seems to be confirmed by what Randerath observed within the tumour tissue in plasmacytoma. Pictures published by this worker show an abundance of paraprotein crystals in the interstitial plasmacytoma nodes, while the lymphatics appear to be filled with a gelatinous mass with absorbing giant cells at its edges. Paraproteins may, therefore, enter the lymph vessels but are precipitated even after having got into their lumen in the same way as it was observed in the renal tubules by Randerath and others.

The phenomenon that paraproteins are accumulated and stored in the interstitial spaces need, therefore, not necessarily be attributed to a primary insufficiency of the lymphatics, nor to the fact that the colloidal molecules are unable to pass through the walls of the lymphatics (it has been noted that also "foreign" substances, such as dextran, polyvinyl etc. are capable of entering the lymph vessels); the phenomenon in question is more probably caused by the fact that the proteins either crystallize in the interstitial space or precipitate in the lymphatics and so obstruct the vessels. Pathology knows many other instances of proteins, once escaped from the capillaries, being precipitated and so being prevented from getting into the lymphatics.

In lobar pneumonia, at the stage of hepatization, fibrinous exudate is present in the alveoli. It is obviously impossible for the lymphatics to carry off such non-liquid exudate. The same fibrinous, coagulated exudate is sometimes observable in the lymphatics of the diseased area. The lymph flow becomes capable of transporting the exudate only after it has been liquified by the leucocytes.

Apart from the facts discussed above, there is a further factor that should be noted. Randerath declared that, in cases of amyloidosis, protein crystals could be demonstrated also within the endothelial cells of the lymph-vessel walls. We had occasion to observe that the endothelium of the lymph capillaries also showed pathologic alterations, at least, certain endothelial cells became conspicuously swollen

in the main of early, fresh cases — no dilated lymphatics indicative of dynamic insufficiency, so that we were probably dealing with an insufficiency of the absorptive apparatus.

We must admit that the causes responsible for the disturbance of protein transportation, for the formation of oedema and a subsequent cicatrization in the discussed cases are still obscure. Also factors other than those mentioned are presumably involved in the development of these processes. For example, the following possibilities present themselves in connection with the development of absorptive insufficiency. We have already noted that lymph capillaries are "anchored" to the fibres of connective tissue and, instead of being compressed, their walls are rather drawn apart by the pressure of possible oedemas. Such "anchorage" may in certain cases cease by a destruction of the fibres concerned. If this happens, any oedema pressure would tend to compress the lymph capillaries and give rise to histological symptoms similar to those observable in glomerulonephritis and intercapillary glomerulosclerosis.

What has been stated in the foregoing applies, essentially, also to alterations observed in the thyroid. Factors of a possible significance in this connection are: mechanical insufficiency of the lymph circulation induced by the adenomatous node and its connective tissue capsule; degeneration of lymph capillary walls; cicatrization of the substance contained in the lymphatics; interstitial inflammation with consequent fixation of the protein to the ground substance of the connective tissue; finally, a change in and a precipitation of the protein, i.e. the colloids of the thyroid gland. Which of these factors plays the decisive role in any given case, still awaits elucidation. We think we have contributed to the solution of the problem by having pointed to the possibility of an insufficiency in the protein and fluid transport and by having discussed the mechanisms that may be involved in the process.

has proved that proteins accumulated in the renal interstices in such cases are actually plasma proteins. Another possibility is an allergic inflammation in the kidney and a "fixation" of the protein in the interstitial space, similar to that we have noted in the skin in connection with Arthus' phenomenon. Still another explanation is offered by the possibility that the lymph-capillary walls become diseased like those of the glomerular capillaries and the pathologic process to cause a reduction of endothelial permeability. We do not regard the latter alternative as very probable, since pathological processes — as has been pointed out above — have the tend-

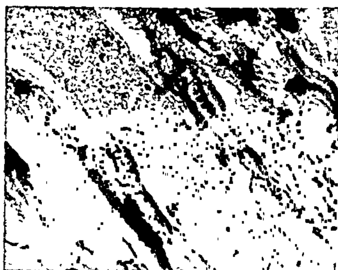


*Fig. 161* Pathologically swollen endothelial cells of lymph capillary in the human thyroid

ency to rather increase than decrease the permeability of the lymphatics in the same way as they do in the case of blood capillaries (glomeruli). It should be borne in mind that Rényi-Vámos and Róna obtained their material from patients who were succumbed to the disease so that their results were all based on the observation of cases where the morbid process was fairly advanced. It is conceivable that increased capillary permeability allows the interstitial space to be flooded by plasma proteins which leads to a dynamic insufficiency of the renal lymphatics. Collagenous fibres may then arise in the unremoved protein both outside and inside the lymph vessels which causes the whole area to become cicatrized and the lymphatics to become obstructed. Similar processes may have occurred in our cases of intercapillary glomerulosclerosis. True, this supposition is weakened by the fact that there were also in our material — one that consisted

TABLE 44

*Composition of transudates (according to C. Schmidt 1850)*

| | Pleural transudate | Peritoneal transudate | Cerebrospinal fluid | Oedematous fluid |
|---|---|---|---|---|
| Water | 96·39 | 97·98 | 98 35 | 98 87 |
| Protein | 2 85 | 1·11 | 0 80 | 0 36 |
| Ashes | 0 76 | 0 98 | 0 85 | 0 77 |
| Spec. weight | 1 013 | 1·011 | 0 009 | 1 007 |

the literature contained rather contradictory data. Therefore, Bálint and Benkő (1948) found it necessary to reinvestigate the repartition of these ions between the extravascular fluids and the blood plasma. They determined in 38 cases the protein, Na and Cl content in the serum on the one side and that in the ascitic, pleural and cerebrospinal fluid on the other; they found that the proportion between the Na-Cl content of the serum and that of the fluids was in good agreement with the value calculated on the basis of Donnan's theory by means of Van Slyke's formula. Hence, it was proved by these investigations that the distribution of the most important electrolytes between *serum and extravascular fluids satisfies Donnan's equilibrium* theory. In accordance with the above, the electrolyte content of the serous fluid is influenced not only by the electrolyte content of the serum but also by the protein concentration of the serum and the extravascular fluids, as the barrier separating the two fluids behaves more or less like a semipermeable membrane.

In their publication, Bálint and Benkő claimed the *protein content* of the ascitic fluid to be 1·1 to 4 8 g% and that of pleural fluid 2·9 to 5·9 g%, but it should be noted that pathological fluid accumulations and frequently inflammatory exudates are also included in these cases.

Maurer, Warren and Drinker (1940) investigated the normal peritoneal and pericardial fluid in dogs, cats and rabbits. The protein content of the peritoneal fluid amounted to 1 63—3·71% (average, 2·56%) in dogs, to 1·53% in rabbits and to 2·52% in cats. The protein content of the pericardial fluid was 0 83—2 88% (average: 1·70%) in the dog, 2·67% in the cat and 3 65% in the rabbit.

Drinker et al. (1940) compared the protein and chloride content of serum, cardiac lymph and pericardial fluid (Table 45) and concluded that, on the one hand, the composition of pericardial fluid and that of cardiac lymph were fairly similar and found, on the other hand, that both the lymph and the fluid of the serous cavities were identical with the extracellular fluid. But inspection of their tables convinces us that — though collected from the same animal — lymph and pericardial fluid are fairly different in respect of protein content, which we

# CHAPTER IX

## FILTRATION AND ABSORPTION THROUGH SEROUS MEMBRANES

Cavities lined with serous membranes are frequently regarded as belonging to the lymphatic system both morphologically and physiologically. These cavities do, as a matter of fact, contain a fluid the composition of which is strikingly resembling to that of lymph. Certain authors spoke, therefore, even recently of pericardial, peritoneal, etc., lymph (e. g. Gerhartz 1925). In the course of decades, numerous data were published to prove that lymph, the fluid normally obtainable from the serous cavities, as also the pathological accumulations of fluid occurring there (ascitic and hydrothoracic fluid) and the oedematous fluid in the subcutaneous connective tissue, had essentially the same composition as the blood plasma, and that they differed from the latter only in their [illegible] with the assumptic [illegible] extravascular fluid [illegible]

Reports on investigations to this effect were very numerous even in early literature. We want to refer here only to Schmidt's classic work (1850) who examined the composition of different transudates in the same person. Such data have, however, the common fault that, as a rule, the composition of pathological (of congestive or inflammatory origin) and non-pathological accumulations of fluid was compared and, besides, the composition of blood plasma and its changes in various diseases (e.g. cirrhosis of the liver) were left out of consideration. The examination of the fluid of the serous cavities is undoubtedly difficult because their amount is very small under normal conditions. Yamada and his associates (1933), for example, were able to draw pleural fluid by puncture from only 29 per cent of healthy persons and the quantity they succeeded in collecting did not usualy exceed one or two drops and was never more than 20 ml. Nor is the amount of fluid generally much more that can be obtained from the pericardial sac or the peritoneum of normal animals and humans.

Peters (1933) claims in his fundamental work on the water content of the organism that the fluid in the serous cavities is suggestive of a protein-free serum filtrate, contains the *same* organic substances as blood plasma and that its electrolyte content corresponds to the Donnan-equilibrium. The serous fluid is, however, not protein-free: all investigators who examined these fluids found in them — as did Schmidt — more or less protein. As to the distribution of electrolytes, there is a difference between serum and serous fluids as regards potassium and calcium content: serum contains somewhat more of these ions (Peters 1935). In respect of the proportion of Na and Cl ions,

TABLE 44

*Composition of transudates (according to C Schmidt 1850)*

| | Pleural transudate | Peritoneal transudate | Cerebrospinal fluid | Oedematous fluid |
|---|---|---|---|---|
| Water | 96·39 | 97·98 | 98·35 | 98·87 |
| Protein | 2·85 | 1·11 | 0·80 | 0·36 |
| Ashes | 0·76 | 0·98 | 0·85 | 0·77 |
| Spec. weight | 1·013 | 1·011 | 0·009 | 1·007 |

the literature contained rather contradictory data. Therefore, Bálint and Benkő (1948) found it necessary to reinvestigate the repartition of these ions between the extravascular fluids and the blood plasma. They determined in 38 cases the protein, Na and Cl content in the serum on the one side and that in the ascitic, pleural and cere-

means of Van Slyke's formula. Hence, it was proved by these investigations that the distribution of the most important electrolytes between serum and extravascular fluids satisfies Donnan's equilibrium theory. In accordance with the above, the electrolyte content of the serous fluid is influenced not only by the electrolyte content of the serum but also by the protein concentration of the serum and the extravascular fluids, as the barrier separating the two fluids behaves more or less like a semipermeable membrane.

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regard as a proof against the identity of the two fluids. Elsewhere, we have already dealt with the question of the identity of lymph and extracellular fluid. Therefore, we propose now to ascertain the relationship between lymph and the fluid of the serous cavities.

TABLE 45

*Protein content of cardiac lymph and pericardial fluid (according to Drinker et al 1940)*

| | Total protein g% | Albumin/globulin ratio | Chloride mg % | Colloid-osmotic pressure mm $H_2O$ | |
|---|---|---|---|---|---|
| | | | | *in total fluid* | *calculated for 1 g of protein* |
| Serum | 5·95 | 1 10 | 416 | 234 | 38 |
| Cardiac lymph | 3 83 | 1·40 | 450 | 175 | 44 |
| Pericardial fluid | 1 63 | 1·34 | 434 | 59 | 43 |

Were the serous cavities really part of the lymphatic system, as was frequently assumed, the identification of serous fluids with lymph would undoubtedly be justified. It is, therefore, very important to ascertain whether there exists a direct communication between lymphatics and serous cavities (peritoneum, pleura) through openings, stomata and stigmata.

Anatomical literature contains many publications concerning the question of peritoneal stomata. V. Recklinghausen (1862, 1863, 1869) was the first to describe direct communication between peritoneum and lymphatics through the stomata in the peritoneum and the stigmata in the wall of the corresponding lymph vessels. Kolossow (1893) denied the existence of permanent openings: he suggested that they appeared only when intraperitoneal pressure increased; this view was shared by MacCallum (1902, 1903). Muscatello (1895), Maximov (1927) and Hertzler (1901) also denied the existence of stomata. Although — relying on the results yielded by his peroxidase technique — Magnus (1922) supposed the existence of a direct communication between lymphatic lumen and peritoneal cavity, the stomata postulated by v. Recklinghausen are nowadays generally regarded as artefacts. Zhdanov (1952), who has studied this question quite recently, accepts the theory of Bizzozero and Salvioli (1878) according to which there are holes — suctorial organs — on the diaphragmatic surface of the peritoneum; though these are not genuine openings, since they are still covered by a mesothelial layer, a close contact does, nevertheless, exist here between pleural cavity and lymphatics. It was Matochkin (1949) in the first place who concerned himself with the function of these holes.

The holes in question perform a pump-like function: when the diaphragm contracts, i.e. at inspiration, they become wider and longer so that the pressure in them is decreased, and they are thus able to aspirate fluid and suspended particles from the free abdominal cavity. At expiration, the holes are compressed, pressure in them increases, and the substances contained in them are squeezed through the mesothelial layer into the lymphatics. We consider it probable that — when this happens — the fluid gains access to the lumen of the lymph channels through the pores in the intercellular cement substance; however, these submicroscopic pores are evidently not wide enough to let larger particles, e.g. red blood corpuscles, pass.

At the end of this short survey of the pertinent anatomical literature, the conclusion seems justified that there exists no direct communication between lymph vessels and serous cavities so that the latter cannot be regarded as parts of the lymphatic system.

## ABSORPTION OF CORPUSCULAR PARTICLES FROM SEROUS CAVITIES

The question remains open as to how corpuscular elements penetrate from the serous cavities into the lumen of lymphatics. It seems most likely that they are driven by external mechanical pressure [illegible] ports which re- [illegible] the following. [illegible] nce of a direct communication between the lymphatics and the peritoneal cavity, the passage of corpuscular particles from the peritoneum into the lymphatics meant no special problem. Seeing, however, that v. Recklinghausen's theory may now be regarded as overridden from an anatomical point of view, we want to concern ourselves only with those views according to which the serous cavities are closed and have no direct communication with the lymphatic system.

Let us first mention Muscatello's hypothesis (1895): he thought that the corpuscular elements were phagocytozed by leucocytes and passed in this condition into the lymphatics through the serous membranes. Simer (1947), too, described that two days after the intraperitoneal injection of an India-ink suspension leucocytes containing India ink were demonstrable in the lymph vessels of the diaphgragm. But these observations do not yet prove that absorption is in fact effected through phagocytosis by leucocytes. Signs of phagocytosis can only be demonstrated after about 30 minutes, whereas — according to MacCallum (1903) — absorption occurs very rapidly: India ink appears in the mediastinal lymph nodes and lymphatics as soon as 5 minutes after intraperitoneal injection.

Simer (1948) demonstrated in the course of more recent experiments that granules of a diameter of less than 5—6 $\mu$ pass through the peritoneum at the edges of the mesothelial cells (in the intercellular

cement) into the efferent lymphatics freely, i.e. without being phagocytozed. Larger particles, e.g. the blood corpuscles of the frog, cannot, in his opinion, be absorbed from the peritoneum of the rat and transported by the lymphatics.

This observation is, however, in contradiction to earlier experimental results which showed that the entire amount of blood introduced into the serous cavities was comparatively rapidly absorbed. Ostrowski (1935), for example, introduced 150 to 250 ml of blood into the pleural cavity of dogs and found that the whole amount was absorbed within 3 to 6 days. Nikolski (1895) administered 300 ml of defibrinated blood intraperitoneally: although practically nothing was absorbed after 5 hours, after a day more than half of the blood was nevertheless removed. This is important also because the question has repeatedly arisen as to what can be expected of intraperitoneal transfusions, in what manner and how rapidly blood is absorbed from the peritoneum and whether this method should be employed in the absence of adequate veins. Florey and Witts (1928) gave a negative answer. Although the infused red blood corpuscles do get absorbed, absorption takes place very slowly: in dogs, for example, only 1/16 of the introduced erythrocytes is absorbed in 6 hours and a great number of red blood corpuscles is encountered in the abdominal cavity even many hours after the injection of blood. In their opinion, absorption is promoted by high intra abdominal pressure (vigorous [illegible]

[illegible]

Phagocytosis by leucocytes could not be observed or was insignificant in any case.

In rats, 70 per cent of the injected erythrocytes, labelled with $^{32}P$, were absorbed within 5 hours (Courtice and Morris 1953; Morris 1953). Absorption in larger animals apparently needs more time. In dogs, for example, only 20 per cent of the erythrocytes labelled with radioactive iron were absorbed in 24 hours (Hahn and his associates 1944). According to Courtice, Harding and Steinbeck (1952), absorption is quickest in rats, slowest in guinea-pigs, while, in rabbits, values lie between these two limits. Hedenstedt (1944) studied the absorption in infants, and found that most of the injected blood was absorbed within 7 to 8 days.

It should be noted that the rate of absorption depends also on the nature of the particles: graphite and India-ink suspension, as also other substances which irritate the serous membranes, are fixed, becoming attached to the surface of the mesothelial membrane so that their absorption is considerably slowed down. Bangham, Magee and Osborn (1953) observed the peritoneal absorption of radio-active glass globules

and amorphous glass particles (diameter: 0.5 to 1.5 $\mu$) in rats and mice. The amorphous particles provoked marked inflammation and, when the injection was repeated after 36 hours, their absorption became significantly decreased. Although the initial absorption of amorphous particles is more rapid than that of glass globules, the total absorbed quantity of the former is less than that of the latter. This is attributed by the authors to the fact that, at the beginning of the inflammatory process, absorption is more vigorous, while — later — the substance becomes fixed to the inflamed peritoneum, however fixation does not take place until inflammation has reached full intensity.

If the corpuscular elements are not transported by phagocyting leucocytes, the question arises: how do they pass through the mesothelial cells of the serous membranes and the lymph capillary walls?

Cunningham (1922a, 1926) having observed in the mesothelial cells India ink, erythrocytes and other granules, thinks that phagocytosis by these cells play a decisive part in the absorption of corpuscular elements. The fact that certain corpuscular elements can be demonstrated also in the endothelial and mesothelial cells does not — in itself — prove that they play an important role in the transportation of these particles. This process is in any case too slow to explain the quick passage of the particles into the efferent lymphatics.

MacCallum's experiments, in which he poured India-ink suspension on the diaphragm of eviscerated dogs and used artificial respiration for a number of hours, seem to us to be very convincing. Microscopic examination showed that India ink was deposited on the diaphragm at the borders of the mesothelial cells; this led MacCallum to the conclusion that the Indiaink escaped from the peritoneal cavity between the cells, i.e. through the intercellular substance. MacCallum's observations were later confirmed by several authors (Florey 1927; Allen 1936; Simer 1948).

Respiration, i.e. movements of the diaphragm, and changes of the intraabdominal pressure exert great influence upon the absorption of corpuscular elements. As has been already pointed out, movement and external pressure are decisive factors of lymphatic absorption and lymph flow.

Above, we have discussed a mechanism in which the movement of diaphragm plays an important role through the function of the peritoneal "suction holes". Florey (1927) pointed to the importance of intra-abdominal pressure. The idea that absorption is promoted by the increase of peritoneal pressure is very old and was advanced by Wegener as long ago as 1877. Relaxation of the abdominal wall (deep [illegible]

duces contraction of the musculature of the abdominal wall) absorption is accelerated. Wegner placed a small rubber balloon between the liver and diaphragm of rabbits and found that, in correspondence with

the respiratory movements, changes in pressure amounted to 3—5 cm $H_2O$ even in deeply anaesthetized animals with relaxed abdominal muscles. In unanaesthetized animals these fluctuations of pressure are surely still greater. Relying on these experiments, he came to the conclusion that changes in the intra-abdominal pressure, by which corpuscular particles are squeezed through the ground substance between the mesothelial and endothelial cells, are decisive for their absorption and that phagocytosis or similar mechanisms probably play only a subordinate role.

We are not so sure that the fluctuations of intra-abdominal pressure have really such a great importance in the transportation of corpuscular elements. It is, however, not to be denied that, somehow, respiratory movements do play an important part. This was proved by MacCallum's experiments (1903) which showed that by artificial respiration, performed on the dead body of the dog, it was possible to make intraperitoneally administered particles pass into the lymphatics. Higgins and his associates (1930) observed that, after unilateral phrenicotomy, absorption on the paralyzed side of the diaphragm became much slower. According to Allen and Vogt (1937), it is not through their influence on the intra-abdominal pressure but by a reduction of intralymphatic pressure that respiratory movements affect peritoneal absorption. Expansion of the diaphragm leads — according to their idea — to a dilatation of the lymph capillaries and consequently to a decrease of intralymphatic pressure so that the capillaries take in fluid and corpuscular elements. In the following phase, at the contraction of the diaphragm, the content of the lymphatic capillaries is then forced into the larger efferent lymph channels.

Recently, Morris (1953) examined the action of diaphragmatic movement on peritoneal absorption and found that the absorption of erythrocytes labelled with radio-active phosphorus and that of protein is considerably diminished by a paralysis of the diaphragm as it is also by deep anaesthesia. Absorption is, on the other hand, considerably accelerated by the inhalation of $CO_2$, i. e. by the acceleration of respiratory movements.

The observed action of diaphragmatic movements on peritoneal absorption led Mengle (1937) to certain practical conclusions. He demonstrated that in ether anaesthesia (which he had found to be the best respiratory stimulant of all anaesthetics used by him) intraperitoneally injected graphite particles appeared in the efferent lymphatics of normal animals very quickly. Absorption was much slower in local or spinal anaesthesia. In local peritonitis or diffuse peritonitis of 1—2 days, the absorption of corpuscular elements was likewise promoted by ether anaesthesia. It is therefore suggested by Mengle that ether anaesthesia should be avoided in cases of inflammatory peritoneal diseases and that one should rather employ an anaesthetic which does not promote diaphragmatic movement and so facilitate the penetration of bacteria and toxic substances into the circulation. Courtice

and his co-workers (Courtice and Simmonds 1949b; Courtice and Morris 1953 and Morris 1953), also, concerned themselves with the effect of anaesthesia in rabbits and rats. According to them, the absorption of labelled red blood corpuscles and protein is diminished by nembutal.

Absorption from the peritoneal cavity depends also on the position of the animal. We have already mentioned Florey's observation (1927) that absorption is accelerated by suspending the animals with their head downwards, whereas absorption is slowed down in the opposite position (i. e. when the body of the animal is beck, with the head upwards). Courtice and Steinbeck (1951) also found that absorption from the abdominal cavity into the lymphatics was diminished when rabbits were in this position. Bangham and his associates (1953) observed that radioactive glass globules were absorbed from the peritoneum of rats at a slower rate when the animals were erect, and at a more rapid one when they were suspended with their head downwards. But this phenomenon cannot be explained — as is done by Florey (1927) — solely by changes in the intraperitoneal pressure. Courtice and Simmonds (1954) are undoubtedly right in assuming that the position of the animal is important for peritoneal absorption because it affects the transport of the substances to the site of absorption. Absorption in serous membranes has been found mainly to occur at certain preferential points.

It has been known since the experiments of v. Recklinghausen that corpuscular elements are absorbed from the peritoneal space principally on the inferior diaphragmatic surface. This question forms the subject of a recent publication of Courtice and Simmonds (1954). Almost all investigators who have studied the question of peritoneal absorption confirm this earlier observation. It seems, however, that absorption is not uniform all over the diaphragm. In rabbits and guinea-pigs, absorption is probably quickest in the region of the central tendon (Brown 1928; Courtice and Steinbeck 1950); this is, however, not the case in dogs, cats and rats (Higgins and Graham 1929; Courtice and Steinbeck 1950, 1951).

Whether also other areas of the peritoneum are, and if so to what extent, involved in the absorption constituted another unsolved problem for a long time. To-day, it is generally accepted that neither the parietal peritoneum nor the mesentery play any important part, at least not quantitatively (Courtice and Simmonds 1954).

Buxton and Torrey (1906) demonstrated that, after intraperitoneal injections of India ink, the lymphatics of the omentum become suffused with the dye in the guinea pig. On the other hand, as we have seen (cf. chapter on the anatomy of the peritoneal lymphatics), it is held by many authors that the omentum contains no lymphatics at all. According to Simer (1944), the lymph vessels of the omentum play a negligible part in absorption. However, Higgins and Bain (1930) and Zhdanov (1940a, 1942a) warn us that omental absorption must not be

disregarded. According to Braude (1948, 1950), the peritoneum covering the coecum and Ligamentum latum is an important surface of absorption.

The absorption of colloids and corpuscular particles from the peritoneum occurs however, above all, on the inferior diaphragmatic surface, and any change in the position of the animal which renders the transport of the substance to the surface of the diaphragm more difficult decreases absorption. True, Courtice and Simmonds (1954) found that it was not wholly impeded even when the animals were in an erect position, with their head upwards. By respiratory and diaphragmatic movements, transport towards the diaphragm of the substances injected into the peritoneum is undoubtedly promoted whatever the position of the animals. In addition intestinal peristalsis may also play a part (Higgins and co-workers 1930).

We want to point here to another very important fact: according to Courtice and Steinbeck (1950), the lymph originating from the lymphatics of the diaphragm is, at least in the cat, transported by the right lymphatic trunk. Recently, it was demonstrated by Courtice, Harding and Steinbeck (1952) that, after the intraperitoneal injection of 10 ml/kg of blood, the number of erythrocytes in the right lymph trunk, increases very quickly and may reach even 4—5 millions per ml. The erythrocytes appear also in the lymph of the thoracic duct, but these, their number does not exceed a few hundred thousands. Courtice and his associates think that, in all probability, the majority of the peritoneal lymphatics is drained by the right trunk also in other animals (dog, guinea pig, rabbit) (Courtice and Simmonds 1954). Abdou, Reinhardt and Tarver (1952) observed that, in rats, about 75 per cent of the intraperitoneally injected plasma (labelled with $^{14}C$) was absorbed by the thoracic duct. Hence, the problem still awaits elucidation.

*The examination of the lymph obtained from the thoracic duct* may, therefore, present a very false picture of the rate of absorption from the peritoneum. For this reason, we cannot attach much value to the results of those earlier experiments in which the role of the lymphatic system in peritoneal absorption was determined by ligation of the thoracic duct with the idea of so blocking the route of substances absorbed from the abdominal cavity through the lymphatic system. On the other hand experiments in which a cannula is tied into the right lymphatic trunk offer many possibilities of error. First of all, the trunk in question is very short and of no constant formation: the vessels of which it consists, are frequently independent of one another and empty separately into the large veins of the neck. It is frequently impossible to introduce a cannula into the right lymphatic trunk of dogs and cats; but even if one succeeds in inserting a cannula into a lymphatic, it remains still doubtful whether it is really the right lymphatic trunk or some other vessel. It was for this reason that, in our own experiments, we examined the lymph of the thoracic duct

for the investigation of peritoneal absorption, although it seems to us possible that — in the dog — it is not the thoracic duct that forms the principal efferent path of the peritoneal lymph. Though no quantitative conclusion can be drawn from the lymph of the thoracic duct as to the magnitude of absorption, changes in absorption can nevertheless be followed by examining that lymph, and one has the added advantage of avoiding the technical difficulties and eliminating all factors of uncertainty connected with the examination of the lymph coming from the right lymphatic trunk.

Absorption of corpuscular particles from the *pleural cavity* has also been thoroughly studied. Respiratory movements play an important role in pleural absorption. Dybkovski (1866) saw stomata between the mesothelial cells of the pleura through which, in his opinion, the corpuscular elements were pressed into the lymphatics by respiratory movements. Introducing India ink and other suspended particles into the thoracic cavity of dead dogs, he observed that, under the effect of artificial respiration, the particles gained access to the pleural lymphatics. Wadsworth (1922), too, considered respiratory movements very important for the absorption from the pleura (India ink, carmine, milk). According to Bettmann (1925), India ink injected into the thoracic cavity of normal animals disappears from it much more rapidly than in animals with artificial pneumothorax. As Dolley and Wiese (1929) have shown, the amount of lymph draining from the thoracic duct decreases by 45 per cent through artificial pneumothorax. We are of the opinion that this has little to do with pleural absorption, since most of the lymphatics of the pleura do not empty into the thoracic duct and also because normal negative intrathoracic pressure is — as has already been pointed out — a very important factor in the maintenance of *lymph flow*.

Courtice and Simmonds (1949b) demonstrated that, in rabbits and cats, the absorption of protein and dye from the pleura was decreased by the diminution of respiratory movements (deep anaesthesia). Recent experiments of Courtice and Morris (1953) have furnished further evidence concerning the role of diaphragmatic movements in pleural absorption. They found that the absorption of erythrocytes labelled with radioactive phosphorus was decreased by bilateral phrenicotomy in the acute phase, while the absorption of protein appeared to have rather increased. In the chronic phase, i.e. 6 weeks after the phrenicotomy, erythrocytes were once more absorbed at the usual rate. Increased respiratory movements (inhalation of $CO_2$) promote, and anaesthesia reduces the absorption of protein and erythrocytes.

Much controversy arose not only in connection with peritoneal absorption but also on the question as to whether there are stomata in the pleura and whether a direct communication exists between the pleural cavity and the lymphatics. It was emphasized by Afanasiew (1868) that in none of the dogs and rabbits examined by him did he find the pleural stomata described by Dybkovski. He suggests that

introduced dye diffuses in the pleural cavity, passes into the subpleural connective tissue and, from there, into the lymphatics.

Miller (1947) affirms in his monograph on the lung that the pleura contains no stomata and that the structures described are nothing but artefacts. In no circumstances do openings develop in the normal pleura. Absorbed particles have to pass through the pleural mesothelium. As regards pleural absorption — the same as in connection with the peritoneum — Drinker and Yoffey (1941) incline to the view of MacCallum that, when the serous membranes are stretched by respiratory movements apertures may arise which are but temporary and invisible at rest.

Rouvière and Valette (1937) attach great importance to the specific reaction of the endothelial (mesothelial) cells, and state that the appearance of intrapleurally introduced India ink, Prussian blue suspended in oil, as well as that of starch grains can be very quickly (after 4 to 5 minutes) demonstrated in the subpleural connective tissues and that they appear in the pleural lymphatics likewise fairly rapidly (after 8 to 9 minutes).

Starch grains of a diameter of 50 $\mu$ are already demonstrable in the submesothelial connective tissue after 8 minutes. The fact, however, that after a certain time comparatively few particles of India ink are visible between the numerous droplets of fat that have gained access to the lymph vessels, is regarded by them as a proof of a specific function of the endothelial cells.

Without intending to offer here a detailed criticism of this theory of Rouvière and Valette, we only point to the fact that the respective amounts of India ink and fat (diluted India-ink solution and diluted olive-oil) administered by them were different and, further, that the India-ink granules may have been retained by the lymph nodes, etc. We do not deny that the properties of membrane (size of pores, electrical charge, etc.) play a very important role in the absorption through biological membranes; yet, we regard the claim of the authors, i.e. that the observed differences are due to a special activity of the endothelial cells, as lacking sufficient foundation.

Also Zhdanov (1940a, b, 1952) is of the view that pleural absorption takes place not through the intercellular substance but the mesothelial and endothelial cells themselves, with their active co-operation.

According to Vitels (1947), pleural absorption goes through such openings as were described in the peritoneum. These holes must not be confunded with the stomata, since the mesothelium is intact even in the "openings" and only the layers of submesothelial connective tissue are absent.

Vitels affirms that these structures — situated mainly in the costal pleura, but also in the retrosternal area and in the pleura which covers the central tendon of the diaphragm — show a particular arrangement.

The question as to at which point pleural absorption occurs, is also a battleground of controversies. Karsner and Swanbeck (1920/21)

suggest that the absorption of granular substances occurs principally through the mediastinal pleura; the visceral and diaphragmatic pleura show less activity, the least active being the parietal pleura which lines the thoracic cavity.

In contrast, Wadsworth (1922) found that particulate elements are principally absorbed by the parietal pleura. Absorption from the visceral pleura is insignificant and is effected by way of phagocytosis, Karsner and Swanbeck also attached great importance to the phagocytosis by mesothelial cells.

In contradistinction to these authors, Iwanow (1939) doubts the possibility of any foreign substance being absorbed from the pleural cavity. Zhdanov (1949b) thought it therefore necessary to re-examine [illegible] from the entire pleural surface and that also colloidal trypan blue stained diffusely the pleura and the subpleural connective tissue. It depends mainly on the position of the animal which part of the parietal pleura will stain most intensively. Collargol, which has a lower dispersity, is absorbed more slowly and mainly through the mediastinal pleura. Absorption, as a rule, is fairly rapid. As early as 5 minutes after the intrapleural injection India-ink particles pass through the pleura and reach the efferent lymphatics. Within 30 to 120 minutes after the intrapleural injection well-stained subpleural lymphatics can be seen, principally below the parietal pleura covering the intercostal muscles. Neither below the parietal pleura covering the ribs and the diaphragm, nor below the visceral pleura did Zhdanov see any lymphatics containing India ink.

It is in any case certain that the inferior diaphragmatic surface plays a decisive role in peritoneal absorption, whereas absorption [illegible] Morris 1953).

According to Courtice and Simmonds (1954), the principal sites of pleural absorption are the inferior mediastinal and costal parietal pleura, although it is not possible to determine the relative significance of these two surfaces.

Drinker and Field (1931) examined the absorption of trypan blue and graphite from the *pericardial* cavity. In their opinion, absorption takes place very slowly, its principal pathway being the lymphatics at the base of the parietal pericardium. Particulate matter is absorbed by way of phagocytosis. This was confirmed by Stewart et al. (1938) as also by Dible and Lynch (1938). However, we do not think it probable that the absorption from the pericardium should take place through a mechanism different from that of other serous cavities concerning which it has been noted that phagocytosis plays no substantial part.

## ABSORPTION OF PROTEIN AND COLLOIDAL MOLECULES FROM SEROUS CAVITIES

No mention has so far been made of the absorption of fluids and dissolved substances for while, since v. Recklinghausen's experiments (1862, 1863), it is clear that particulate matter is absorbed by the lymphatics, the way in which water, dissolved crystalloids and colloids are absorbed has remained unelucidated.

*According to Ludwig's filtration theory, the absorption of fluids* which have passed into the connective tissues and serous cavities through filtration or other means is performed exclusively by the lymphatics. However, not even Orlow's early results (1895) could be brought into harmony with the theory that fluid which has gained access to the abdominal cavity is simply absorbed towards the lymphatics in an unchanged condition. Orlow's experiments showed that the peritoneal absorption of fluids depends also on their composition.

Orlow found that hypertonic and hypotonic solutions, introduced into the peritoneal cavity, became isotonic with blood plasma. As regards hypotc
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in the abdominal cavity. No essential change in the lymph flow of the thoracic duct occurs either during the rapid absorption of hypotonic solutions, or in the course of the slower absorption of isotonic saline solution or homologous serum, or after the intraperitoneal administration of hypertonic solution.

*Orlow drew two conclusions from his results: first, that diffusion* from and into the blood plasma constitutes an important factor in the absorption of fluids introduced into the peritoneum and, second, that peritoneal absorption requires the active, vital participation of the cells of the serous membrane.

Although — criticized from the angle of our present knowledge — Orlow's experiments and conclusions can no longer be regarded as satisfactory, they were of fundamental importance, as it was he who first demonstrated that the absorption of fluids and dissolved substances does not invariably occur through the lymphatics, and that the possibility of a resorption by the blood capillaries also exists.

Starling and Tubby (1894) came to similar conclusions. Having injected dyestuffs (carmine and methylene blue) into the pleural cavity, they observed that the dye always appeared earlier in the urine than in the lymph of the thoracic duct. Solutions of protein were, on the
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blood capillaries; with regard to the slower absorption of proteins and other colloidal substances they thought it probable that these, as also corpuscular elements, were absorbed by the lymphatics.

Chittenden, Mendel and Henderson (1899) as well as Dudrey (1899) confirmed the experimental results of Orlow, Starling and Tubby according to which blood capillaries are involved in the absorption of fluids from serous cavities.

Exner (1903) suggested that substances whose peritoneal absorption is inhibited by adrenaline are taken up by the blood vessels, while substances resistant to adrenaline are absorbed by the lymphatics.

The investigations of Watkins and Fulton (1938) yielded nothing essentially new. They stated, as had Orlow, that intraperitoneally administered saline solution, 6% gum-arabic, horse serum and blood did not increase lymph flow in dogs. They affirmed that the amount of fluid absorbed by the lymph vessels from the peritoneum is insignificant. Gum-arabic is absorbed comparatively best by the lymphatics: according to the calculations, about 24 per cent of the missing amount. However, the experimental results of Watkins and Fulton cannot be regarded as reliable for they, too, collected the lymph from the thoracic duct only and disregarded the right lymphatic tunk.

Notkin (1925) studied the absorption of saline solutions, colloids and corpuscular particles from the peritoneum in the dog, rabbit, cat and rat. He found that the introduced crystalloids ($K_4 FeCN_6$, $NaNO_3$) appeared more quickly in the urine than in the lymph of the thoracic duct. As regards the absorption of (hypertonic, hypotonic and isotonic) saline solutions, Notkin confirmed Orlow's results.

More interesting are Notkin's investigations concerning the absorption of colloidal solutions (haemolysed blood, egg albumin); he found that haemolysed blood is absorbed but slowly from the abdominal cavity. After the intraperitoneal injection of 100 ml of haemoglobin, it appeared in the urine after about 3¼ to 4 hours, whereas even 6 to 8 hours after the administration 28 to 78 ml of haemolysed blood still remained in the abdominal cavity of the animals. No haemoglobin was encountered in the urine of those animals in which the thoracic duct had been tied off: this, however, does not indicate the absence of absorption, since two days later, when the animals were killed, only 20 to 30 per cent of the injected fluid was found to have remained in the abdominal cavity. According to Notkin, though these experiments show that protein absorption is not prevented (but only slowed down) [illegible]

[illegible] tion of protein solutions from the peritoneum is still possible after ligation of the thoracic duct.

Dissenting from this concept, Shipley and Cunningham (1916) refuse to acknowledge the assumed participation of the lymphatics in the peritoneal absorption of colloidal substances. Colloidal dyes which they had administered intraperitoneally in the course of their experiments, appeared promptly in the liver. They suggest that the

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Orlow found that hypertonic and hypotonic solutions, introduced into the peritoneal cavity, became isotonic with blood plasma. As regards hypotonic solutions, at first only water is absorbed from them at a rapid rate, whereas hypertonic solutions are diluted by diffusion of water from the blood plasma so that the amount of fluid increases in the abdominal cavity. No essential change in the lymph flow of the thoracic duct occurs either during the rapid absorption of hypotonic solutions, or in the course of the slower absorption of isotonic saline solution or homologous serum, or after the intraperitoneal administration of hypertonic solution.

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into the circulation). The authors had previously furnished reliable evidence that the dye was quantitatively adsorbed to the plasma proteins under their experimental conditions, and that proteins were absorbed together with the dye so that the dye level of plasma and lymph could be accepted as a measure of protein absorption. They hold that dye cannot pass from the pleura into the circulation unless through the lymphatics. The same applies to the proteins absorbed from the peritoneum (Courtice and Steinbeck 1950, 1951). However, some absorption of dye and protein from the peritoneal cavity is traceable in the blood sometimes even after the ligature of the right lymph trunk and the thoracic duct.

They observed further that the absorption from the thoracic cavity of the dye T-1824 is promoted by the presence of protein (blood plasma). This observation is quite in harmony with our own results concerning diffusion in connective tissues and absorption of dyes through lymphatics. Courtice and Simmonds explain this phenomenon as we do, i.e. by the fact that the dye dissolved in saline solution is adsorbed to tissue proteins, whereas dye dissolved in plasma, attached to protein as it is, becomes absorbed together with the same and cannot be arrested in the tissues.

Courtice and Simmonds (1949b) did not determine the amount of the dye removed by the lymphatics but only the increase in the plasma concentration, a method that, in our opinion, is not always adequate. We examined, therefore, the absorption of a protein-bound dye from the abdominal cavity under experimental conditions similar to those of Courtice and Simmonds (Szabó and Magyar 1954). We, too, administered the dye diluted with plasma respectively, with physiological saline solution.

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[illegible] tant factors of peritoneal and pleural absorption. In anaesthetized animals, lying on their back, respiratory movements and lymph flow are diminished (Drinker 1945). The second objection would be that the efferent lymphatics of the peritoneum do not empty into the thoracic duct but into the right lymphatic trunk (Courtice, Harding and Steinbeck 1952).

We do not think that these two objections are sufficient to invalidate our experimental results, for absolute values are less important in the given case than the differences between the results of the two groups of experiments. Also, we have already pointed to the difficulties and disadvantages associated with the collection of lymph from the right lymph trunk.

Results are illustrated in Fig. 162. It shows the respective amounts of lymph (in ml) and dye (in $\gamma$) drained per minute after the intraperi-

dye was absorbed by the blood *capillaries of the omentum and passed* from there into the portal vein. This theory was supported by Poynter (1928), and rejected by Higgins and Bain (1930). The data of the last-named authors cannot be accepted as pertinent because they used a graphite suspension and not colloids.

According to Loeschke (1931), colloidal dyes are absorbed from the serous cavities through special perivascular paths, and it is from them that the dyestuff reaches the lymphatics.

Zhdanov (1910b) confirmed Loeschke's results in so far as he found the surroundings of the veins intensively stained by colloidal dye introduced into the peritoneum. Zhdanov denies, however, that this staining is in any way connected with the absorption of colloids and that it points to the presence of some perivascular cleft. In his opinion — which we are inclined to share — all that is happening here is the adsorption of dyestuffs to the fibres of the connective tissue. In essence, we have here the phenomenon that had already been described by Anitschkow in 1924 in connection with the intravenous administration of trypan blue: it, too, stains the fibres of the connective tissues in the whole body, and the perivascular reticulum of elastic fibres in particular.

Zhdanov succeeded, moreover, in directly demonstrating the transport of colloidal dyes through the lymphatics. In his experiments, well-stained diaphragmatic lymphatics could be seen not later than 5 minutes after the intraperitoneal injection.

Colloidal dyes and other colloids (e. g. collargol) are, therefore, absorbed principally by the lymphatics, but Zhdanov does not dismiss the possibility that the absorption of these substances may occur — partly at least — through the mesenteral capillaries.

Samuelsen et al. (1948), after having drawn a large amount of blood from dogs, studied the regeneration of the plasma proteins. They found the initial increase of the plasma protein level to be quicker after the intraperitoneal re-injection of the blood than in animals into which the blood had not been re-infused.

Recently, Courtice and his associates re-examined the absorption of protein from serous cavities. In their first publication (Courtice and Simmonds 1949b), they observed the absorption of 0·9% saline solution and of blood plasma from the pleural cavity. They observed that physiological saline (6 ml/kg) and plasma were absorbed from the pleural cavity at approximately the same rate and that both disappeared in about 24 hours. Protein labelled with T-1824 is — according to them — exclusively absorbed by the lymphatics, dye and protein passing into the circulation principally via the right lymphatic trunk, which is proved not only by the fact that more dye is drained by this trunk per unit of time than by the thoracic duct but also by the observation that the concentration of the dye in the plasma does not rise in animals with fistula of the right trunk or in those in which this lymphatic is tied off (i.e. when the content of the trunk cannot pa-

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absorption of the protein-dye complex is diminished by acute and chronic phrenicotomy as well as by anaesthesia, but increased by the inhalation of $CO_2$. Absorption of colloids from the pleural cavity is decreased by narcosis, but perhaps rather promoted by acute and chronic phrenicotomy. Inhalation of $CO_2$ also produces a marked increase in pleural absorption.

Besides respiratory movements and other mechanical factors, presumably also other factors play a part in the absorption of colloids through serous membranes. Orlow (1895) assumed that active cellular functions, the "vital functions" of the mesothelial and capillary endothelial cells, too, were involved in peritoneal absorption. He based this assumption on the observation that peritoneal absorption was markedly impeded by fluoride.

Some of our experimental results seemed to support this assumption. Let us refer here to our experiments in which we studied the lymphatic transport solutions containing a protein-dye complex injected into the abdominal cavity of dead animals.

We isolated and inserted a cannula into the thoracic duct of dogs; this done, the animals were killed by excessive doses of the anaesthetic. We then injected into the abdominal cavity fluid of the same composition and amount as in the preceding series of experiments (25 mg/kg of Congo red dissolved in bovine serum diluted at the rate of 1 : 1). Artificial respiration was maintained after the death of the animals by means of a Starling's pump through a tracheal cannula.

The results of this series of experiments are illustrated in Fig. 162/III. It can be seen that — under equal conditions — substantially less of the intraperitoneally administered dye was absorbed in dead than in living animals (Fig. 162/II).

This result seems to be in contradiction with the statement made by us in connection with peripheral absorption. Let us refer to the experiments in which the absorption of subcutaneously injected dyes was investigated in living and dead animals: it was observed that the dye appeared in higher concentration in the efferent lymphatic of the dead than in that of the living animals. We concluded that the permeability of the lymphatics increased after death. How then may we explain the contradictory phenomenon observed in the present cases? Are perhaps peritoneal lymphatics different in nature and behaviour from peripheral lymph channels? We do not think such assumption necessary. It should be remembered that, in the case of peripheral absorption, the injected dye — after its diffusion in the connective tissue — has to pass through a single membrane only, i.e. the lymph capillary wall, whereas the intraperitoneally introduced protein-dye complex has to overcome one more barrier, i.e. the peritoneum. It is possible that, after death, the permeability of the mesothelial membrane changes in the opposite direction, that is, its permeability may postmortally decrease. This may be interpreted by the assumption that dye and protein are adsorbed to the connective tissue more vigorously after death than during life. This would be in harmony

toneal injection of 40 ml/kg of physiological saline solution (I) and of diluted bovine serum (II). *The dispersion of the results is fairly great,* but it is nevertheless quite evident that the amount flowing from the thoracic duct per unit of time is higher in the case of dye administered with protein than in that introduced with physiological saline. It is,

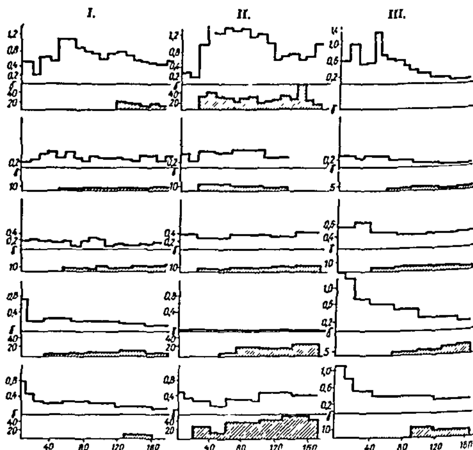



*Fig. 162.* Lymph flow in the thoracic duct (ml/min.) and dye secretion (γ/min) after intraperitoneal injection of 25 mg/kg of Congo red

I The dye dissolved in 40 ml/kg of physiological saline II Dissolved in 40 ml/kg of diluted serum III The same in the dead animal

therefore, true that the presence of protein increases the lymphatic absorption of dyes (in this case that of Congo red). On the other hand, this effect is demonstrable not only in respect of pleural but also in that of peritoneal absorption.

It has been mentioned that Courtice and his associates proved the absorption-promoting effect of respiratory movements also in connection with protein. According to Morris (1953), the peritoneal

to 24 hours in guinea pigs. The maximum rate of transport per hour is estimated by the authors at 25 per cent of the volume of circulating plasma in rats and 10 per cent in rabbits.

Serum seems to be absorbed more slowly from the pleura than from the peritoneum. Pleural absorption of 6 ml/kg of serum took 8 to 16 hours in rats (Lake, Simmonds and Steinbeck 1953) and 24 hours in rabbits (Courtice and Simmonds 1949b).

According to Hamburger (1902), *serum and physiological saline* solution injected into the *pericardium* are absorbed at the same rate [illegible] involved. According to Gorinstein (1913), it is by way of the lymph vessels that India ink and dyestuffs are removed from the pericardium. The lymphatics of the pericardium communicate with those of the mediastinum which explains the spread of pericardial inflammatory processes to the mediastinum.

In some instances, Drinker and Field (1931) observed the absorption of homologous and heterologous sera from the pericardial sac of rabbits. They claim that absorption is very slow, but it is emphasized in a recent report of Courtice and Simmonds (1954) that further, more extensive researches are required before a definite attitude can be taken in this matter.

As a matter of fact, a great many problems concerning protein absorption still require elucidation. The fluid to be found in the serous cavities under normal conditions — but also pathological accumulations of fluid — always contain more or less protein; even if protein-free fluid is injected into the serous cavities, it will be found to contain protein after some time because protein diffuses from the blood capillaries into the unabsorbed fluid. Why is this protein not carried away by the lymphatics? Together with protein, also fluid is evidently absorbed by the lymphatics. What are the factors that influence the amount of this fluid and the protein concentration of the lymph draining from the serous cavities? What, then, are the factors that determine the protein level of the fluid which is contained in the serous cavities under normal conditions?

It is not yet possible to answer all these questions definitely. We believe, however, that we are in a position to give at least a general answer to the last question. Protein is continually diffusing from the blood plasma — which is richer in protein — into the peritoneal and pleural fluid and is continuously removed therefrom by the lymphatics, so that the protein level of the serous fluid essentially reflects the dynamic equilibrium between the amount of protein which has escaped from the blood path and that which is removed by the lymph. That these amounts of protein are very considerable will be discussed in a later part of this work. It was shown by Schoenberger and his associates (1953) that, on an average, 77 g of albumin, i. e. almost $\frac{3}{4}$ of the

with our observation that diffusion is slower in dead than in living animals.

However, also other explanations can be offered. In order that the colloids, injected into the peritoneum and diffused through its wall, might be removed by the lymphatics and pass into the thoracic duct, a good many additional conditions must be fulfilled. It has already been pointed out that active contractions of the diaphragm constitute an important motor of lymph flow (Jossifov 1914, 1930) which is undoubtedly absent in the dead bodies of the experimental animals in spite of artificial respiration. Other important factors in lymph flow are the active functioning and the contractions of the lymph vessels which, too, cease after death. The observed phenomena may, hence, be explained also by changes in the lymph flow, so that the results need not necessarily lead to the conclusion that it is the peritoneal absorption which undergoes profound changes. Consequently our experiments do not prove a participation of cellular activity in the peritoneal absorption of proteins (although this cannot, of course, be excluded altogether). At any rate, available data disprove the existence of such an active transport mechanism, and our observations are explainable also without this assumption.

Courtice and Simmonds (1954) hold that, since the absorption of colloids and corpuscular elements from the serous cavities occurs almost exclusively through the lymphatics. After intraperitoneal or intrapleural administration of the substances in question, their appearance in the blood plasma enables us to draw a direct conclusion as to the rate of absorption. We do not quite agree, however, with this statement. First, notwithstanding the rather convincing experiments to which we shall revert later, the question as to how far the passage of colloidal molecules into the blood capillaries is due to diffusion, does not seem to us to have been definitely elucidated. Secondly, a part of the colloids, as also part of the corpuscular elements, disappears from the circulation irrespective of whether the colloidal molecules have reached it from a serous cavity directly or have gained access to the blood circulation through the lymphatics. As to particulate matter, phagocytosis by the reticulo-endothelial cells seems to be the decisive factor, while colloids escape from the blood stream in the same way as do plasma proteins. Thus, a part of the colloidal molecules absorbed *from the serous cavities disappears from the circulation after* a relatively short period. It is, therefore, not admissible to draw conclusions as to the amount of absorbed colloids from the determination of plasma concentration.

The other statement of Courtice and Simmonds, viz. that the lymphatic system may remove considerable amounts of protein from the serous cavities, is, however, incontestable. The data published by Courtice and Steinbeck (1950, 1951) are these: 20 ml/kg of serum (about half the volume of circulating plasma) are absorbed from the peritoneum in 3 to 5 hours in rats, in 5 to 8 hours in rabbits, in 16

Róth (1899) further examined the absorption from serous cavities in rabbits after ligature of the thoracic duct. He introduced the substances in molar solutions, since their osmotic activity essentially depends on their molar concentration (an unusual physiological concept at that time). Róth, too, found that the volume of the hypertonic solution increased in the first hours, though this could not be by filtration but only by "osmotic water flow". He reached the conclusion that the membrane through which osmosis takes place is less permeable to dissolved molecules than to water. Whether the diffusion of water outwards through the capillary wall will predominate or the penetration of dissolved molecules into the capillaries, depends on the permeability of the membrane (capillary wall and serosa) to the molecules in question. As Róth observed, simultaneously, molecules not contained in the original solution will diffuse from the blood, i.e. the partial osmotic concentrations are also equalized.

Róth demonstrated furthermore that the capillary endothelium is differently permeable to different molecules, the differences depending also on molecular size. Only carbamide is an exception: it is absorbed from the peritoneum in equimolar (equiosmotic) concentration more quickly than saline. Róth refers to Lazarus–Barlow (1895/96), Leathes (1895/96) and others who, after intravenous administration, observed a similar sequence in the disappearance of various substances from circulation.

In connection with this phenomenon, Korányi (1897) declared that the "exchange of molecules" was not based on the vital function of the separating cellular layer but induced by physico-chemical forces.

We can see that Korányi and his disciple had strikingly modern conceptions in this domain, also. Their findings were far ahead of their time by pointing to the physico-chemical basis of the processes involved in the peritoneal absorption of water and dissolved molecules. Many of the more recent experiments, performed with up-to-date methods, yielded only rediscoveries of this or that forgotten finding of Korányi a [illegible] sults of Putnam (1923), Schechter [illegible] thers.

Recent [illegible] studied the absorption of fluid, salt, glucose and amino acids administered intraperitoneally and subcutaneously to guinea pigs. Isotonic saline solution (about 50 g/kg) disappeared from the abdominal cavity within 15 hours, whereas isotonic solution containing 2.5% glucose and 0.45% saline took up water from the blood plasma during the first 7 hours, decreased to its original volume only after about 12 hours, and was not fully absorbed even after 24 hours.

The concentration of Na and Cl in the peritoneal fluid rapidly became equal to the concentration which is encountered in the plasma. The explanation of this phenomenon is evident. Diffusible water and electrolytes penetrate quickly into the peritoneal cavity, equilibrium on both sides of the peritoneal membrane is promptly established,

whole amount of circulating albumin, pass, in man, through the peritoneal membrane alone, every day. Do the lymphatics really transport such a considerable amount of protein and the amount of fluid which goes with it? This question also belongs to the group of unsettled problems, the solution of which possesses great practical significance beside theoretical interest.

## ABSORPTION OF WATER AND CRYSTALLOID MOLECULES FROM SEROUS CAVITIES

The experimental results discussed above have made it clear that while proteins and other colloids which have gained access to the serous cavities are mostly absorbed by the lymphatic apparatus, the absorption of water and crystalloids is performed mainly by the blood capillaries. We want to examine this question briefly by referring to the fundamentally important work of Róth (1899), who was the first to point to the most important factors of the process of absorption.

The absorption of fluids from serous cavities is described by Ellinger (1902) who, in doing so, relies on the works of Orlow (1895), Starling and Tubby (1894), Hamburger (1895), Conhstein (1896a), and — *above all — on that of Róth. The essential points of Ellinger's* views can be related as follows: After the intraperitoneal injection of hypertonic saline solution, the latter takes up water from the blood plasma so that its volume is increased and its concentration decreased. The salts absent from the original solution diffuse at the same time into the peritoneal fluid from the blood. After the intraperitoneal injection of a hypotonic fluid the process is reversed: the fluid first becomes concentrated by the absorption of water towards the circulation, whereupon it is absorbed like isotonic solutions. Isotonic solutions remain, however, isotonic throughout and only their composition changes: it becomes similar to that of blood plasma. The absorption of isotonic solutions is due to the colloid-osmotic pressure of blood proteins (Cohnstein 1896a; Starling 1896). Solutions with protein content also first become isotonic with blood plasma, and so much water is then absorbed from them by the blood capillaries that the *protein content and the colloid-osmotic pressure* of the peritoneal fluid become equal to those of the blood plasma (Róth 1899). Thereafter, further absorption occurs through the lymphatics alone.

It should be noted that this theory of Róth has to be somewhat modified according to recent results reached by Courtice and Steinbeck (1951). Experimenting on rats, they observed that, after intra-
ein became diluted in the
the original protein level
*an equilibrium*, the value
of which is lower than the protein concentration of the blood plasma: it amounts to about 3—5 g per cent.

3. As a local factor, predisposing to the appearance of ascites, portal congestion i.e. an increase in the capillary filtration pressure is added.

4. Another factor responsible for the development of ascites is the insufficiency of the lymphatic system: the lymphatics are unable to remove the excessive amount of transudate from the abdominal cavity.

5. Disturbance in the renal excretion of sodium, probably due to haemodynamic and endocrine factors (increased secretion of aldosterone).

The following question might be raised in this connection: what is the cause of the insufficiency of the lymphatic system postulated by us in this case? An occlusion of the lymph vessels, which would account for their mechanical insufficiency seems very improbable; nor have we reason to suppose a primary disturbance of fluid absorption. So nothing but a dynamic insufficiency of the lymph circulation seems to explain the phenomenon. There is no doubt that the causes set out above (decreased colloid-osmotic pressure, increased capillary pressure, etc.) lead to increased filtration. The transporting capacity of the lymphatic system is, however, limited so that it is not able to remove all the amount of filtered fluid and proteins.

Schoenberger and his co-workers (1953) carried out experiments on normal individuals and on cirrhotics with ascites in order to ascertain the variations of "endothelial permeability" in these cases. After the intravenous or intraperitoneal administration of albumin labelled with radioactive iodine, the authors determined the time needed for the equilibration of the radioactive protein and also determined the amount of protein passing through the membrane per unit of time. Dynamic equilibrium was found to exist between intravascular and extravascular albumin. According to their calculations, the average amount of albumin exchanged through the peritoneal membrane after intravenous injections amounted to 3·2 g (2·2—4·6 g) per hour in normal controls and to 3·4 g/hour (1·05—7·10 g) in cirrhotics. Equilibration between blood plasma and ascites fluid after the intraperitoneal injection of labelled albumin takes comparatively little time in cirrhotic patients (48 hours) even in the injection is given at a time of rapidly increasing ascites fluid (after paracentesis) (Schoenberger et al. 1952). It would follow from these experimental results that the rate of transperitoneal protein transport remains unchanged in cases of hepatic cirrhosis, which means that — provided the protein that has passed from the capillaries into the peritoneum is carried off by the lymph vessels — lymph flow is not increased at all in cirrhosis so that we cannot speak of dynamic insufficiency. The experiments of Eisenmenger and Slater (1953), performed with a similar method, led to similar results. Prentice and his collaborators (1952) pointed out that ascites fluid should not be regarded as a stagnant pool, for in experiments with heavy water they had succeeded in demonstrating that 40 to 80 per cent of the ascites fluid were exchanged within an hour.

while glucose does not diffuse so easily, so that equilibration is a slower process. As long as the concentration of glucose in the peritoneal fluid remains higher, while the concentration of electrolytes is already in equilibrium, peritoneal fluid will absorb water from the blood plasma. This is proved by the fact that the absorption of the fluid introduced into the peritoneum begins only when its glucose level has become equal to that of blood plasma. These phenomena are still more marked after the intraperitoneal injection of hypertonic glucose or amino-acid solutions.

We have already referred to earlier experiments, which showed that intraperitoneally and intrapleurally injected dyes of small molecular size appear earlier in the urine and blood than in the lymph; they led to the conclusion that crystalloids are mainly absorbed by the blood capillaries. Rehn (1913) performed similar experiments regarding percardial absorption. Dye injected into the pericardium (indigo carimine) stained the urine even after 5 minutes, whereas much more time was required for its passage into the mediastinal lymphatics. Rehn pointed to the fact that water and crystalloids were absorbed also from the pericardium towards the blood capillaries.

## ROLE OF THE LYMPHATIC SYSTEM IN THE ORIGIN OF SEROUS EFFUSIONS

The serous cavities, as has been noted, contain only a few millilitres of fluid in normal circumstances, while — under certain pathologic conditions — significant accumulations of fluids may arise (ascites, hydrothorax, hydropericardium). A decisive role is ascribed in the origin of these accumulations to increased capillary filtration and to inflammatory exudation. We are, however, justified also in this case in raising the question: why is the excessive amount of filtered fluid not removed by the lymphatics? The question is the more justifiable as it is known that lymphatics are capable of removing intraperitoneally or intrapleurally injected protein-rich fluids fairly rapidly. It must, therefore, be assumed that in all cases where fluid accumulates in the serous cavities lymphatic drainage becomes insufficient for some reason. Unfortunately, only very few pertinent experiments are known to us; these, however, seem to support our assumption.

It is in hepatic cirrhosis that the largest accumulations of fluid in the abdominal cavity have been observed. It cannot be doubted that, in these cases, a number of factors contribute to the formation of ascites. In one of our publications (Szabó and Littmann 1952) we made an attempt to summarize these factors as follows:

1. Colloid-osmotic pressure decreases in the blood serum as a result of disturbed protein (especially albumin) production.

2. Failing to be excreted by the diseased liver, antidiuretic hormone of the posterior hypophyseal lobe is retained. These two factors promote, as a rule the formation of oedema.

After inducing experimental ascites, McKee and Stewart (1950) examined also the absorption of erythrocytes labelled with radioactive iron. Whereas, in normal dogs, these were completely absorbed in 72 hours, only 26, 39 and 51 per cent, respectively, of the erythrocytes (contained in 75 ml of blood) were absorbed in the three test animals during the phase of rapid ascites formation. Absorption was more rapid during the phase of *slow ascites formation*: 43, 56 and 67 per cent, respectively, were absorbed in 72 hours.

These results of McKee and his co-workers would justify the conclusion that in ascites the peritoneal absorption of protein and red blood corpuscles, i.e. the lymph flow, though not stopping altogether, decreased in any case. This conclusion does not seem to be acceptable. The introduced radioactive substance surely became diluted by the great amount of ascitic fluid (the abdominal cavity of the animals contained 1 to 2 litres of fluid) so that it must have reached the outflowing lymph in a low concentration. It was, on the other hand, noted by the authors that in these chronic experiments (the animals were examined through 11 months) signs of chronic inflammation and adhesions were found in the peritoneum when the animals were autopsied: processes of this kind are known to lead to a reduction of absorption.

Bollman et al. (1950) and Nix et al. (1951) performed direct observations of the lymph flow in dogs during the formation of ascites. They found a tremendous increase in the amount of lymph originating from the liver in experimental cirrhosis after the constriction of the inferior vena cava cranially from the hepatic vein. While, in normal animals, daily ½ to ¼ of the total circulating plasma protein is transported by the lymphatics of the liver, the amount of protein removed by the liver lymph is 3—4, even 10-fold of the whole amount of circulating protein in cirrhotic animals or in cases of venous congestion of the liver.

Bolton's earlier experiments led to similar results. It was found also by him that the absorption of colloidal dyes from the abdominal cavity did not stop after supradiaphragmatic constriction of the inferior cava, and that, after ascites had been formed, the lymph flow increased in the thoracic duct.

Nix, Flock and Bollman (1951) studied the changes of lymph flow in the rat under the influence of hepatic cirrhosis. After inducing cirrhosis by means of carbon tetrachloride, they found that the amount of lymph draining from the Cisterna chyli increased 6 to 7-fold, and the amount of transported protein 8-fold.

It seems to be clear from these experiments that, in hepatic cirrhosis, the filtration of fluids increases and that greater amounts of fluid and protein are transported by the lymphatics. Of course, these data point to an increased production of liver lymph in the first place, but it is beyond doubt that the formation of intestinal lymph may also be enhanced by portal congestion. The amount of fluid and protein which the lymphatics are unable to remove because of their dynamic

A closer scrutiny of these data will make it apparent that they are based on erroneous conclusions, for it is quite obvious that the above-mentioned transcapillary or transperitoneal exchange of 3·2 to 3·4 g of albumin cannot be effected through the filtration or absorption of fluid. It would mean that about 100 ml of plasma per hour are filtered into the peritoneum and the same amount returned to the circulation by the large lymphatics (by the right lymphatic trunk in the first place). According to available data it is, however, improbable that, under normal conditions, the lymphatics are able to transport such a great amount of protein and water from the peritoneum. Nor can it be supposed that the albumin is reabsorbed by the blood capillaries, since there seems to be no force capable of maintaining the process of capillary reabsorption of proteins. The huge exchange of albumin must evidently be due to diffusion, that is to say that, both in normal individuals and in cirrhotic patients, there exists a constant diffusion of protein through the pores into the peritoneal fluid and from there back into blood capillaries, and that this process is independent of filtration flow. It is independent not only of the filtration of fluid brought about by capillary pressure but independent also of the absorption of fluid and protein through the lymphatics.

McKee and his associates (1950a, b; 1952) investigated peritoneal absorption in experimental ascites. It is known that it is very often possible to induce ascites in a short time by the supradiaphragmatic constriction of the inferior vena cava of dogs (McKee and associates [illegible] injection, 35 per cent of the labelled protein was found 48 hours later in the plasma, whereas further 22% had disappeared from the ascites without appearing in the plasma. In the after intravenous introduction, 40 per cent of the labelled protein had disappeared from the ascites in 48 hours but not more than 10 per cent appeared in the plasma. 70 hours after the intravenous administration they found 30 per cent of the labelled protein in the ascites and 12 per cent in the plasma, so that 58 per cent had disappeared in the extracapillar protein depots. In other experiments, equilibrium between ascitic fluid and blood [illegible] ises [illegible] itic [illegible] ys) than that of globulin (2 or more days). According to the calculations of the authors, the transperitoneal transport of albumin is at least three times as rapid as that of globulin. This result is in contradiction to the statement of Courtice and Simmonds (1954) that absorption of protein molecules through the peritoneal membrane is not essentially hindered so that albumin and globulin are absorbed at about the same rate.

ies (Parker 1951). The observation, made in some cases, that ascites isappears after pleural paracentesis seems to confirm this possibility. The phenomenon could also be explained by the assumption that — s a consequence of the aspiratory effect of restored negative intra horacic pressure — the lymphatics transport the fluid from the perioneum into the pleural cavity. However, it is conceivable that there eally exists in such cases an anomalous communication between the wo cavities.

Hydrothorax associated with ascites is not an exclusive feature of Meigs' syndrome; accumulation of pleural fluid is frequently found lso in ascites caused by hepatic cirrhosis. According to Lichtmann (1949), in cirrhotics ascites is associated with hydrothorax in 1 to 3 per cent of the cases, whereas it is in 3·5 per cent of ovarian tumours that ascites is accompanied by hydrothorax ("Meigs' syndrome" 1954).

Mechanisms operative in cases of ascites due to cardiac decompensation are essentially the same as in hepatic cirrhosis: venous and portal congestion; disturbance of water and salt balance caused by complicated mechanisms; increased capillary filtration which exceed the transport capacity of the lymph channels. However, in this instance not only a dynamic but also a mechanical insufficiency of the lymphatic apparatus has to be considered. As already mentioned, in cases of cardiac failure where central venous pressure is higher, congestion may spread over to the lymph vessels and prevent their drainage into the large veins which will increase filtration from the lymphatics on the one hand and disturb the lymphatic transport of fluid and protein from the abdominal cavity on the other.

In connection with the origin of pleural fluid accumulations, Végh, Kocsár and Kertész (1957) pointed out some interesting features. Clinical experience shows that thoracic effusions due to cardiac failure occur more frequently on the right side, while those of hypoproteinaemic, nephrotic origin are more frequent on the left side. They examined, therefore, the absorption of radioactive semi-colloidal bismuth lactate from both sides of the thoracic cavity. In normal dogs, the total amount of colloid administered to the right side was absorbed within 3 hours, whereas only half of the introduced substance disappeared from the left side. In plasmapheresis, on the other hand, absorption was considerably more rapid from the left side in four cases out of five. Since the right side of the diaphragmatic pleura is much richer in lymph channels than the left side (Matotchkin 1949), the authors assume that the divergences in the localization of phlebohypertonic and hypalbuminaemic pleural transudates must be due to some special disturbance of the lymph flow. In phlebohypertension, when congestion extends to the lymph vessels, insufficiency of the lymphatic system is more marked on the right pleural surface which is larger and better vascularized. In hypoproteinaemia, however, when lymph production is strongly increased, the larger lymphatic apparatus of the right-hand

insufficiency diffuses through the peritoneum into the free abdominal cavity; a number of reports suggest that the fluid passes from the lymphatics themselves (principally from those of the liver capsule) into the abdominal or even the thoracic cavity as a result of increased intralymphatic pressure. According to Volwiler (1951), the lymphatics of the hepatic capsule and hilus are extraordinarily dilated and engorged with fluid in cases of ascites produced by the constriction of the inferior thoracic portion of the superior vena cava. The ascitic fluid produced in such cases has a very high protein level which amounts to about 60 per cent of the protein content of the plasma. Therefore, we think it is possible that this fluid passes really through the wall of lymphatics into the abdominal cavity (Bolton and Bernard 1931). This theory was confirmed by the observations of Halmágyi and Robicsek (1954). Hyatt (Hyatt and Smith 1954; Hyatt 1955) observed, in dogs, in the presence of ascites provoked by the constriction of the inferior vena cava and a saltrich diet, a considerable leakage of fluid from the surface of the liver capsule. The protein content of this fluid was equal to that of the liver lymph and the blood plasma.

It has already been pointed out by us that fluid and protein which have gained access to the lymphatics may again diffuse from or be filtered through them. Similar phenomena have repeatedly been observed also in connection with peritoneal absorption. It was, for instance, observed in rabbits and guinea-pigs that — after the intraperitoneal injection of plasma — lymph began to ooze from the parasternal lymphatics into the mediastinum and the thoracic cavity (Courtice and Steinbeck 1950, 1951). If the corresponding lymph nodes are tied off, the lymph pressed into the lymphatics is filtered through the wall of the larger vessels which induces a massive oedema in the mediastinum and a free accumulation of fluid in the pleura (Courtice and Steinbeck 1951).

Worthy of note in this connection is Meigs' syndrome which — though recognized in gynaecology only since the report of Meigs and Cass in 1937 — had been described by several authors at the end of the last century. According to Meigs (1954), all cases belong to this group in which solid ovarian tumours (fibroma, granulosa-cell tumour, theca-cell tumour, Brenner tumour) are accompanied by ascites and hydrothorax. It is here of secondary importance how the fluid arises, whether it is secreted by the tumour cells or brought about by the oedema, the hydropic degeneration of the tumour; of primary importance is the fact — proved by Meigs — that the fluids in the abdominal and the thoracic cavities are identical. So the question arose as to how it was possible for the fluid to gain access to the pleura from the peritoneum. The answer is quite easy bearing in mind the observation of Courtice and Steinbeck: the fluid gets from the extended efferent lymphatics into the thoracic cavity in a secondary manner. Another — very controversial — possibility would be that in these cases a direct communication exists between the thoracic and abdominal cavi-

and thrombosis of the portal and mesenteric veins. So, beside disturbed lymph flow, a venous congestion causing increased filtration was present. *Neuenkirchen (1890) described a bilateral chylous hydrothorax* and attributed it to a sclerosis and rupture of the thoracic duct. Martin (1890) observed in the thoracic and abdominal cavities an accumulation of chylous fluid which accompanied thrombosis of the *left subclavian vein. In the cases* published by Senator (1895), Leydhecker (1893), Weiss (1894) and Fehr (1931), the thoracic duct was closed by a tumorous mass. In Talalajeff's case (1927), on the other hand, almost *all* larger lymphatics, *save the thoracic duct*, were occluded by endothelial proliferation; peripheral oedema, chylous ascites and hydrothorax were present. Fränkel (1892) found a left-side chylous hydrothorax associated with proliferative lymphangitis of the thoracic duct, as well as chylous hydropericardium. Quincke (1875) and Heppner (1934) published cases concerning the rupture of the thoracic duct caused by trauma. Frankenthal (1931) published in *connection with one of his own cases a voluminous bibliography regarding* cases of chylous ascites described in the literature. In his opinion, its most frequent causes are malignant tumours forming metastases in the lymphatics and lymph nodes, or else, swelling of the lymph nodes of tuberculotic or other origin which obliterates the lumen of the lymph channels. In Frankenthal's own case, a carcinoma starting from the head of the pancreas formed metastases in the abdominal and thoracic lymphatics and lymph nodes: they had become obstructed to such an extent that the thoracic duct was completely empty and collapsed.

A catheterization of the left half of the heart by direct ventricular *paracentesis may traumatize the thoracic duct and chylothorax may* arise (Noble 1958).

Everhardt and Jacobs (1938), on the basis of 69 cases, drew up the following table concerning the aetiology of chylothorax:

TABLE 46

| | |
|---|---|
| Trauma | 25 cases |
| *Tumorous compression of thoracic duct* | 13 « |
| Thrombosis of left subclavian vein | 4 « |
| Tumors of thoracic duct | 9 « |
| Lymphangitis perforans | 2 « |
| Aneurysm-like dilatation of thoracic duct | 2 « |
| Thrombosis of thoracic duct | 1 case |
| *Occlusion of abdominal portion of thoracic duct due to* mesenteritis | 1 « |
| Filariasis | 1 « |
| Unidentified | 9 cases |

pleural surface is more capable of meeting increased demands so that transudates will always appear first on the left side.

## ASCITES CHYLOSUS AND CHYLOTHORAX

*In certain circumstances even a primary disease of the lymphatic apparatus may give rise to accumulations of fluid in the abdomen or the thoracic cage (ascites chylosus, chylothorax).*

"We call chylous ascites accumulations of fluid in the peritoneal cavity, the fluid has a yellowish white colour, a milky appearance, and reveals under the microscope very delicate elementary particles but only the presence of very few cells" — wrote Hirschler and Buday (1889a, b, c) in their report published from the clinic of Professor Frigyes Korányi. They wrote further this: "This rare form of ascites is caused by the penetration of the content of the lacteals into the peritoneal cavity either by diffusion through the wall of the lymph vessels or by the interruption of their continuity or of that of the thoracic duct."

Hirschler and Buday give the detailed history of a case in which repeated abdominal punctures had to be performed; on every occasion 6 to 7 litres of a milky fluid were drawn in which 3·4 per cent of protein and almost 0·5 per cent of fat were demonstrable. Autopsy revealed a diffuse carcinosis of the peritoneum. Histological analysis convinced Buday that the case in question was not one of true carcinoma but of a tumour originating from the endothelium of the lymph vessels. In contradiction to contemporary French authors who doubted that the fluid in the abdominal cavity really arose from the lymphatics in cases of chylous ascites, it was demonstrated by Hirschler and Buday that the fluid was really chyle: the accumulated fluid was poor in cells so that the lipids present therein could not originate from desquamated, fatty degenerated tumour cells, besides, the fat concentration of the ascites increased threefold after the ingestion of fatty meal.

*Chylous ascites and hydrothorax have been mentioned in* literature for a number of centuries. De Diemerbroeck (1685), for example, described chylous ascites and hydrothorax in an individual who had succumbed to kicks and blows. At autopsy, he found that large lymphatic trunks had been ruptured as a result of the trauma. In a case of Morton (1689), the thoracic duct, compressed immediately before its juncture with the left subclavian vein by a mass of lymph nodes, became disrupted. In a case of Hoffmann (1740), the thoracic duct was damaged by a stab with a knife. Ormerod–Wilks (1868, cit. Bargebuhr 1895) observed chylous transudation as a result of thrombosis of the left subclavian vein. Noteworthy also is the case of Renvers (1890) who — at the autopsy of a patient with chylous ascites — found that the thoracic duct was distended and had a tortuous course. The subclavian vein was found to be obstructed by a thrombus at the mouth of the thoracic duct; he also diagnosed cirrhosis of the liver

lymph or chyle is filtered from the lymph channels or, if their wall is ruptured, streams freely into the pleural cavity and the lung parenchyma.

## ABSORPTION THROUGH SEROUS MEMBRANES IN INFLAMMATION

Infections of the serous membranes always involve considerable danger for the whole organism because their large absorptive surface facilitates the rapid absorption of bacterial toxins. It is, therefore, important to ascertain what possibilities of absorption from the serous cavities exist in cases of acute and chronic inflammation.

Notkin (1925) provoked peritonitis by the intraperitoneal injection of 10 to 30 ml of 1% iodinepotassium iodide solution. He found that in the acute stope, after the intraperitoneal injection of haemolyzed blood, haemoglobin did not appear in the urine of the animals within 7 hours but only after 20 hours (under equal conditions, haemoglobin appears after 3½ to 4 hours in normal animals). 16—18 days after the administration of iodine, haemoglobinuria manifests itself within about 7 hours. In similarly induced pleuritis the absorption of haemoglobin was likewise delayed. These results are, however, not quite reliable, as the absorption of haemoglobin was found to be normal by Notkin in cases of peritonitis provoked by *tincture* of iodine.

According to Miller (1938), the absorption of serum globulin, egg albumin, and capsular polysaccharides of bacteria from the abdominal cavity of rabbits is delayed by inflammation (aleuronat, staphylococci, etc.). Being more diffusible, egg albumin shows this effect less than globulin. The absorption of glucose is rather accelerated. Phenol red introduced into the inflamed peritoneum, appears as quickly in the urine as in normal animals. Phenol red and bromophenol blue (but not trypan blue) are more rapidly absorbed from inflamed than from normal connective tissues. Miller concludes that the absorption of the less diffusible proteins, polysaccharides and dyestuffs is delayed by inflammation, while that of more diffusible dyes and carbohydrates rather accelerated.

It was observed in connection with peripheral absorption that substances transported by the lymph vessels were more slowly absorbed from inflammatory than from normal tissues. Conditions in the peritoneum are, however, somewhat different: the fluid is there in contact with a very large absorptive surface.

There is presumably also a difference between absorption in the early and that in the later stages of inflammation. Bangham, Magee and Osborn (1953) demonstrated that radio-active amorphous glass particles which provoke peritonitis by peritoneal irritation were first absorbed more quickly than non-irritative glass globules, while — later — the absorption of the globules became more rapid. In turpentine peritonitis of rats, the absorption of glass particles had not significantly changed

In cases where the accumulation of chylous fluid is added to the rupture of large lymphatic trunks, pathogenesis needs no explanation. How then does the chyle gain access to the thoracic, abdominal and even pericardial cavity if the thoracic duct is occluded but its wall intact?

According to Most (1917), chylous ascites cannot be produced by lymph congestion alone, for its production also requires tumour-cell infiltration of the lymphatics or of the wall of the lymphatic channels, since lymph vessels are not ruptured otherwise. However, Blalock, Cunningham and Robinson (1936) produced in animal experiments chylothorax in 50 per cent of their cases by a ligation of the superior vena cava above its juncture with the azygos. We too observed repeatedly that after ligature of the efferent lymphatics fluid may accumulate in the thorax and the abdomen with or without a rupture of the lymph vessels.

Highly interesting in this connection is the report of Hirschler and Buday (1889a, b, c) in which they write the following:

"Frigyes Korányi explained the origin of chylous ascites in his lectures by saying that a rupture of the lymph vessels causes chyle to be admixed with the intra abdominal fluid, for it is inconceivable that so much endothelium should be turned into fat within a single day as to produce a change of this extent in the composition of abdominal fluid." (It is known that certain anatomists attributed chylous ascites to the fatty degeneration of the endothelial cells.) "...If no interruption in the continuity of the mesenteric lymphatics is encountered, one might explain the entry of chyle into the peritoneal cavity by assuming that it is effected by a leakage through the wall of the wide lymph channels... also v. Recklinghausen and Kraus admit the possibility that chylous ascites may arise through a transudation of chyle alone."

[illegible] may [illegible] et al. which we are in a position to confirm. Let us also refer to all that has been written in the foregoing regarding diffusion through the lymphatic wall.

Delarue, Depierre and Roujeau (1950) published a very interesting case in which — apart from chylothorax — widespread lymphangiectasis and chylous pneumonia were found in both lungs. Reinhardt (1953) saw a similar case some years later. Delarue and his associates indicate a thrombosis of the left subclavian vein and the thoracic duct as the causative factor in their case. According to Reinhardt, the damage was caused by the transfer of the congestion from the obstructed thoracic duct to the right bronchomediatinal trunk through the anastomoses between these two lymph channels. The further course of the process seems to be evident to them: the lymphatics become dilated in both lungs, even a retrograde flow become possible in them;

TABLE 47

*Dextran excretion in the thoracic-duct lymph following intraperitoneal injection of 0·6 g/kg of dextran dissolved in 40 ml/kg of physiological saline*

| No. | Normal control | | | | No. | After 15 mg/kg of dibenamine | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1h | 2h | 3h | Total | | 1h | 2h | 3h | Total |
| 1 | 0 | 3·0 | 8·2 | 11·2 | 1 | 0·1 | 1·4 | 3·1 | 4·5 |
| 2 | 7·6 | 8·1 | 11·5 | 27·2 | 2 | 6·2 | 21·1 | 15·9 | 43·2 |
| 3 | 1·4 | 9·4 | 14·7 | 25·5 | 3 | 5·3 | 16·7 | 25·7 | 47·7 |
| 4 | 5·9 | 9·1 | 15·4 | 30·4 | 4 | 7·3 | 5·7 | 24·4 | 37·4 |
| 5 | 0 | 3·0 | 6·0 | 9·0 | 5 | 0 | 4·8 | 7·0 | 11·8 |
| 6 | 25·6 | 46·6 | 19·9 | 92·1 | | | | | |
| 7 | 0 | 2·0 | 3·3 | 5·3 | | | | | |
| 8 | 5·2 | 30·1 | 43·5 | 78·8 | | | | | |
| 9 | 0 | 6·8 | 28·6 | 35·4 | | | | | |

| No. | Dogs with peritonitis | | | | No. | Peritonitis + 15 mg/kg of dibenamine | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1h | 2h | 3h | Total | | 1h | 2h | 3h | Total |
| 1 | 74·9 | 103·9 | 89·2 | 268·0 | 1 | 27·2* | — | — | — |
| 2 | 9·5 | 9·0* | — | — | 2 | 27·5 | 30·4* | — | — |
| 3 | 98·1* | — | — | — | 3 | 10·8* | — | — | — |
| 4 | 37·8 | 25·0 | 54·5 | 117·3 | 4 | 25·5 | 5·7 | 6·5 | 37·7 |
| 5 | 8·8 | 19·5 | 52·4 | 80·7 | 5 | 60·0 | 36·3* | — | — |

* — perished

that peritoneal absorption is not decreased by inflammation (Table 47).

The evaluation of the experiments with dibenamine was still more difficult as peritonitic animals treated with dibenamine died after 1—2 hours.

Since no further results could be expected from this experimental method because of the great dispersion and the uncertainty of the procedure, we had to content ourselves with the conclusion that peritonitis does not reduce the absorption of foreign colloids from the abdominal cavity towards the thoracic duct. However, we have already pointed to the fact that the most important efferent lymphatics of the peritoneum do not empty into the thoracic duct and that the latter transports only a fraction of the substances injected into the abdominal cavity. It is, therefore, quite conceivable that the amount of dextran drained by the principal efferent path, viz. the right lymphatic trunk, is considerably less than normal.

an hour after the administration of turpentine, while the rate of absorption became (very moderately) reduced after the lapse of 24 hours. Similar results were obtained in mice.

Numerous factors may contribute to the reduction of peritoneal absorption during the later phases of inflammation, e.g. a local fixation of bacteria (Opie 1929), secretion of fibrin on the peritoneal surface or occlusion of lymphatics fibrinous thrombi (Menkin 1931a); adhesions of the peritoneal surfaces; etc.

Conditions are, as can be seen, fairly intricate. Reported data are contradictory. For this reason, we felt induced to re-examine the problem of the absorption of colloidal substances from the peritoneal cavity in inflammation.

In these experiments (Szabó 1954), we examined the absorption of a foreign colloid, dextran. In connection with the investigations concerning the transperitoneal migration of radio-active albumin (Schoenberger and co-workers 1953) it was pointed out by us that the process in question could not consist in a total return of the peritoneal proteins into the circulation through the lymph vessels alone but that a part of the proteins that had gained access to the peritoneal cavity must have found its way to the circulation by diffusion through the wall of blood capillaries. This is still more likely to occur if foreign colloids (e.g. bacterial toxin) pass into the peritoneum, the partial osmotic concentration of which is much higher outside the blood capillaries (in the peritoneal carity) than inside them. Reports in the literature contradict this assumption but are, at the same time, contradictory *inter se*. This fact induced us to use in our experiments not homologous colloids (plasma proteins labelled with dyestuffs or isotopes) but a foreign substance, the absorption of which promised to yield clearer results.

[illegible] collected from them in the same manner.

We also investigated the effect of dibenamine on peritoneal absorption in normal animals as well as in those with turpentine peritonitis. We proceeded in these experiments in the same manner as in the first series, but the animals received 10 mg/kg of dibenamine before the injection of dextran. This experiment seemed to us necessary because we had assumed that in inflammation a spasm of the lymphatics might arise which could have affected peritoneal absorption, and dibenamine was expected to release this spasm.

Results were rather uncertain because of their great dispersion, but even these — comparatively few — experiments allow the conclusion

thoracic duct neither in normal nor in peritonitic animals. It is possible that absorption is really enhanced in other phases of the peritonitis, in its earliest stages and that it is actually reduced in the presence of large adhesions or in cases of great fibrin secretion on the peritoneal surface, although no considerable decrease in absorption appeared in the experiments of Bangham and his associates. Therefore, in contradiction to earlier reports, we should not expect either "fixation" of bacterial toxins or some kind of "protection" from peritonitis, at least not during its acute phase when there is surely no significant reduction in the rate of absorption.

In the following group of experiments we repeated, therefore, the previous experiment, but instead of ascertaining the amount of dextran removed through the thoracic duct, we determined the dextran concentration in the plasma 4; 7 and 24 hours after its intraperitoneal injection. Results are illustrated in Fig. 163. Dispersion is rather great also in this series of experiments but it can be seen that, essentially, values for the normal (9 experiments) and those for the peritonitic dogs (11 experiments) are in agreement, nor did statistical analysis reveal significant differences between the two series ($p = 15\%$, $45\%$ resp. $90\%$). It is, therefore, safe to claim that the absorption of foreign colloids from the abdominal cavity is not significantly reduced even

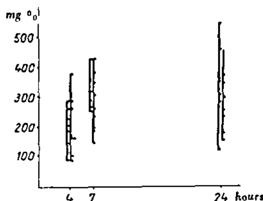



*Fig 166.* Dextran concentration in the blood plasma after intraperitoneal administration of 1·2 g/kg of dextran in the normal and the peritonitic dog

by acute peritonitis so that the danger of an absorption of bacterial products and toxins from the inflamed abdominal cavity is very considerable.

That absorption through the lymph vessels is reduced in peritonitis does not seem to be proved either. Though we investigated only the thoracic duct which is less important from the point of view of peritoneal drainage, we think we are justified in assuming that, whether the removal of colloids is reduced by fibrinous secretion, i.e. lymphatic thrombus, or by spasm of the lymphatics, both these phenomena are bound to occur also in lymphatics drained by the thoracic duct. Accordingly, in inflammation also the thoracic duct would have to drain less dextran from the abdominal cavity. As we have not observed this in our experiments, we are compelled to conclude that the amount of dextran removed by the lymphatics does not decrease in peritonitis, i.e. that the lymphatic drainage of colloidal substances from the abdominal cavity is unchanged. Dibenamine did not significantly affect the transport of intraperitoneally injected dextran through the

animals to various concentrations of salt solution or to open air was capable of deferring their death.

Foglia and Gerschman (1939) studied the composition of blood, lymph and tissues in animals whose lymph hearts had been destroyed, and found a decreased circulating plasma volume, haemoconcentration and also *increased haemolysis as consequences of the intervention. The erythrocytes showed a lower chloride, sodium and potassium content. Haemolysis was attributed by them to diminished concentration of sodium and chloride in the blood plasma.* A reduction of blood volume is accompanied by haemodynamic alterations, such as a drop of arterial pressure and a disturbance of capillary circulation. Diuresis is markedly reduced. Even the composition of tissues undergoes a change in such conditions. Muscles, for instance, lose potassium and chloride, while their water content becomes higher.

Disturbed restoration of protein and fluid in the circulatory system increases the volume of lymph. Extravascularly trapped fluid is comparatively rich in protein (2.05 %). The principal consequence of a destruction of the lymph hearts is that plasmatic fluid, escaped from the blood capillaries, is retained in the lymph sacs and lymph vessels.

Foglia and Zwemer (1943) found that the body weight of frogs *deprived of lymph hearts did not change when kept in air (while — as has been pointed out — it increased by daily 20 per cent in water). In spite of unchanged weight an internal redistribution of the body fluid* was observed. A high degree of haemoconcentration ensues and haematocrit reading goes up very markedly. Uptake of water from the humid milieu is followed by a marked drop in the concentration of plasma proteins. On the other hand, tissues of frogs kept in a dry milieu increase their water content at the expense of plasma water. These authors arrive at the conclusion that a destruction of the lymph hearts checks the normal circulation of plasma and tissue fluid. Inability of the fluids to return to the blood vessels provokes a *shock-like* condition.

*It should be noted that lymphatic retention, a general blockage of lymph flow, may give rise to a serious disturbance of water and salt* metabolism not only in the amphibia but in the mammal as well, a phenomenon studied by Földi, Papp, Solti and Koltay (1957). (See Chapter XII.)

The function of batrachian lymph hearts is the subject of numerous reports. Their contractions were graphically registered by Brucke (1906), Langendorff (1906), Hennequin (1935), Hennequin et al. (1935), Lubsen (1936), as also by Foglia and Braun-Menendez (1939 a, b; 1940): *it is stated by the last-named authors that the contractions of the lymph hearts are not exactly rhythmical and that the principal cause of arrhythmia is a difference in the duration of diastoles.* There is, as a rule, no such difference in the length of systoles. Arrhythmia frequently causes the contractions of the posterior lymph hearts to become asynchronous; such irregularity spreads later to the anterior

# CHAPTER X

# LYMPH FLOW

So far, we have followed the path of the fluid from its filtration through the capillaries, through the interstitial space, up to the point where it reaches the lymphatic capillaries. We have discussed filtration and diffusion through the capillary membrane, the process of spreading in the connective tissue, as also the absorption of fluids and dissolved substances in the lymphatic capillaries. Having thus considered in detail the production and formation of lymph we shall have to examine what happens to the fluid once it has penetrated into the lymphatics, since this is the fluid known as lymph.

Lymphatics constitute a system of efferent ducts through which fluid that has passed into the lymph capillaries from the interstitial tissue is carried off. The question we are facing is this: how do lymphatics perform this work of transportation, what are the factors which govern lymph flow in this system of efferent channels?

## FUNCTION OF LYMPH HEARTS IN AMPHIBIA

In the lymphatic system of lower vertebrates there is a special apparatus maintaining a constant flow of lymph. The destruction of these organs, the lymph hearts, leads in amphibia to loss of fluid from the circulation and progressive haemoconcentration (Abel and Turner 1914; Joseph 1914/15; Isayama 1914 and Ito 1926). Conklin 1930, after destroying the posterior pair of lymph hearts in frogs, observed a reduction of blood volume and temporary oedema in the surroundings of the destroyed lymph hearts. Paralysing of the lymph hearts by means of curarization leads — as has been mentioned earlier in this work — to increased body weight of frogs placed in water and Ringer's solution when injected into the animal, is retained *(mainly in the lymph sacs).*

The investigations concerning the function of lymph hearts, made in the institute of Braun-Menendez, are also worthy of note. It was demonstrated by Foglia (1939) that frogs whose anterior and posterior lymph hearts had been destroyed by cauterization died, as a rule, within 4 days. The rate of mortality reached 87 per cent during the first two days. The body weight of the animals increased very rapidly: it amounted to 20 per cent on the first and 40 on the second postoperative day. In the main, such increase must be due to retention in the lymph sacs and the following fluid-uptake from the surrounding water. The survival of but a single intact lymph heart suffices to keep the animals in an apparently normal condition. No transfer of the

of 2 to 10 mv. This frequency is considerably higher than those reported in the literature (see Table 48).

TABLE 48

*Frequency of contractions of the frog's lymph hearts (from Braun-Menendez and Foglia, 1940)*

| Contractions/min | Author |
|---|---|
| 40—80 | v. Brücke 1906 |
| 10—60 | Winterstein, 1925 |
| 50—60 | Conklin, 1930 |
| 86 | Wakai and Makoto, 1935 |
| 40—70 | Frédéricq, 1936 |
| 50 (average of 38 frogs, at 19 °C) | Foglia and Braun-Menendez, 1939 |
| 80—120 | Papp et al., 1957 |

The wide range of the results is probably related to differences in experimental conditions and may be principally due to differences in the ambient temperature.

Decapitation of frogs was observed by Papp et al. to have been followed by a changed course of the normal action curve, the appearance of arrhythmia and a changed amplitude of the oscillations. They ascribe these phenomena to reflexes elicited by pain, for the amputation of one of the upper limbs of unanaesthetized animals produced similar effects. Complete destruction of the spinal cord was followed by a sudden drop of the rate of oscillation: the lymph hearts stopped after a short time, and a fibrillation curve was developed. Contractions of the lymph hearts were almost synchronous with those of the heart chambers, but the described electric manifestations of the lymph hearts were observed to continue for some time even after the excision of the heart.

Lymph hearts, composed of striated muscle elements, are of course under the influence of the nervous system. As in the case of skeletal musculature, faradic stimulus applied either directly to the lymph heart or to the motor nerves may tetanize the lymph heart (Priestley 1878). Denervation, effected by a section of the nerves or a transplantation of the lymph heart, causes the musculature of this organ to assume properties similar to those of the myocardium. As soon, however, as the nerves are regenerated the musculature of the lymph heart recovers the properties characteristic of striated muscles. The phenomena observed by Papp and his collaborators after the destruction of the spinal cord have been often described in literature. As long ago as 1884 Volkmann observed the reappearance of con-

pair of lymph hearts so that the rate of contraction of the two pairs becomes identical once more. A destruction of one of the posterior lymph hearts does not affect the functioning of the other member of the pair.

As long ago as 1858 Claude Bernard observed the asynchronism of lymph hearts. Pratt and Reid (1932), Hennequin et al. (1935) and Lubsen (1936), on the other hand, point to the synchronism of lymph hearts of the same side, a synchronism which stops as soon as the spinal cord is severed between the fourth and fifth segment.

Foglia and Braun-Menendez (1939) claim that the rate at which the lymph hearts of the Bufo arenarum Hens are pulsating rises, in accordance with Van't Hoff's law, hand in hand with temperature in the range between 0 and 37°C. The effect of temperature on lymph hearts was studied earlier by Winterstein (1925), Lubsen (1936), Ogata, Morita et al. (1933) and others. It was on the lymph hearts themselves or the spinal centres that the last-named authors dripped Ringer's solution of various temperatures. The temperature of the solution rose from 0 to 10 and 15°C. Applied to the centres, it slowed down the pulsation of the lymph hearts in correspondence with rising temperature. A further rise of temperature was followed by increased rate of pulsation. When the temperature of the solution reached about 50°C it began to slow down again. If applied directly to the lymph hearts, the temperature of the solution has no effect on the rate of pulsation; however, the amplitude of contractions increases between 0 and 35°C, decreases between 35 and 50°C, and the lymph heart *suddenly stops functioning when the latter limit is reached*. It was found also by Hotovy (1939) that the temperature of the solution did not affect the rate of pulsation if it was directly applied to the innervated lymph hearts. The frequency of the beat of denervated, automatically functioning lymph hearts responds to even the slightest changes of temperature. Foglia and Braun-Menendez (1939b), in their above-mentioned experiments, submerged the whole animal in water of different temperatures and found that, under such conditions, the rate at which the innervated hearts pulsated went up parallel with temperature: it was — on an average — 15 per minute at 0°C, 61 at 20°C, 100 at 30°C and 123 at 37°C.

The activity of the frog's lymph heart is associated with an action current (Ludány 1929; Brücke and Umrath 1930). Electrical phenomena connected with frog lymph hearts were studied in our Institute by Papp, Zádory, Solti and Holló (1957). Isolating the dorsal lymph hearts of male bullfrogs, they inserted a thin copper electrode beneath them and attached the indifferent electrode to the sacral muscles of the opposite side. The electrodes were then connected to an ECG-apparatus. They simultaneously registered the action potential of the heart. They were thus able to obtain a curve of the normally functioning lymph heart which showed a rhythmical monophasic action current of a frequency of 80 to 120 per minute and a voltage

are able to resume their activity, seems to prove that these organs are more or less autonomous. Even an isolated lymph heart of the frog is capable of functioning during a few hours if placed in Ringer's solution. Moore (1901) and Reid (1933, 1937) transplanted lymph hearts under the tongue, and most of them remained active there. Seeing that the majority of authors is in agreement as to the impossibility of demonstrating the presence of ganglionic cells in the lymph hearts the autonomous contractions are, presumably, of myogenic origin. *The origin of normal rhythm is, however, a problem that has not yet fully been solved.* Normal rhythm is held by certain authors *to be a consequence of the automatism of the lymph hearts which is just modified by the nervous centres. Again, other workers are of the view that normal rhythm originates from the nervous centres* and that automatism comes into play only under abnormal conditions, when nervous connections are interrupted, *as a sort* of compensatory mechanism. Braun-Menendez and Foglia (1940) incline to the latter alternative in their concluding report.

To some extent, also hormonal influences may be involved in the regulation of activity lymph-hearts. Hypophysectomy and adrenalectomy diminish — according to Foglia and Braun-Menendez (1939b) — the frequency of lymph hearts.

Pharmacological agents, such as curare, erythrin, acetylcholine and nicotine, have a paralyzing effect on lymph hearts if injected into the lymph sacs or lymph spaces, or applied locally. Adrenaline, on the other hand, acts as stimulant. Strychnine and veratrine produce no effect unless administered in doses which elicit symptoms of general intoxication. Local administration of these drugs, as also that of ouabain, diminishes the amplitude of contractions. Foglia and Braun-Menendez (1939b) found pilocarpine, atropine, ergotamine and pituitrin to produce no significant effect on lymph hearts.

## MOTORS OF LYMPH FLOW IN THE MAMMAL

As has been shown, the lymph vascular system of the lower animals is provided with lymph hearts, which contribute to the maintenance of lymph propulsion. Experiments quoted in the foregoing make this clear: if, for instance, the activity of the frog's lymph hearts is stopped by the administration of curare, the animal will die because nearly all the circulating plasma gets pooled in the interstitial space or the dilated lymphatics which are not able to return it to the blood vascular system.

Mammals have no lymph heart. How then is lymph propelled in their lymphatics? Of course, lymph flow depends, among other things, on lymph production: on the amount of fluid entering the lymph capillaries. The fluid which has gained access to these capillaries produces a pressure, a push, which may well ensure a uniform lymph flow and the drainage of the lymphatics. The usual argument adduced

tractions some time after the destruction of the spinal cord. This observation has, since, been confirmed by many reports (Goltz 1863; Moore 1901; Bonnet 1934; Pratt and Reid 1930). Contractions in such cases are very weak at first, gradually become stronger, but their amplitude never reaches the original value (Hennequin 1935) Lymph hearts after destruction of the spinal cord are no longer synchronous. This cessation of synchronism is due to the annihilation of the motor centres. Motor centres regulating the movements of the anterior pair of lymph hearts are, according to Ogata and Morita (1937), between the third and fifth spinal segments, while those governing the movements of the posterior pair of lymph hearts are situated at the height of the sixth and eighth rib. These centres are bilateral so that each lymph heart has its separate motor centre. All these centres are governed by a common bulbar inhibitory centre (Bonnet 1934). The function of the latter is shown by the fact that, once it is extirpated, no stoppage of the lymph hearts by reflex actions (e.g. by a blow on the abdomen — Goltz 1863; Moore 1901) can be effected. There exists a close connection between the homolateral spinal lymph hearts as is proved by the synchronism of the homolateral anterior and posterior lymph hearts.

In the main, the innervation of lymph hearts is supplied by the spinal nerves, as also by the nerve fibres which accompany the vessels. A section of the nerves causes a temporary stoppage of the lymph hearts (Goltz 1863; Winterstein 1925; Pratt and Reid 1930; Bonnet 1934; Reinmüller 1935; Lubsen 1936 etc.) which, after some time, begin to function anew. Investigations made hitherto are not sufficient to decide whether one is dealing with an automatism of the lymph hearts or a regeneration of the nerve fibres, since the renewed activity of the lymph hearts lasts a few hours according to some authors and several months according to others. It is, therefore, quite conceivable that it is a regeneration of the nerve fibres which makes such revival of the lymph hearts possible. It has already been mentioned that a faradic stimulation of the nerve endings induces a tetanus of the lymph hearts. Isolated stimuli elicit isolated contractions. Sympathetic stimulation seems to produce an inhibitory effect (Reinmüller, 1935).

Summing up their opinion regarding the influence of the nervous system on lymph hearts, Braun-Menendez and Foglia (1940) wrote the following: "Under normal conditions the nerves are those which command the contractions of the heart by making them arrive the impulsions issuing from the medullary motor centers and also from other centers of reflex orders. The presence of the inhibitory fibres is not so sure. As for the ways which follow these excitements it is essentially the accessory (spinal) nerves III and IV — above all the IIIrd — for the anterior heart and the IXth especially by its ventral limb for the posterior heart."

The fact that not even after denervation and a destruction of the spinal cord do lymph hearts cease to function or, rather, that they

that dyes contained in them travel to a distance of 10 to 15 cm within the space of 5 minutes. Thus, according to these authors, there is a lymph flow in the skin even if it is at rest. It is, however, stated by them that "lymph flow" or rather the rate at which the column of injected dye advances, depends on the amount of injected fluid, i.e. on the injection pressure. Zhdanov (1952)—after injecting radio-opaque substances (collargol, thorotrast) into the subfascial and intermuscular clefts, of cats, dogs and rabbits—observed that the radio-opaque matter advanced 5 cm in about 15 to 20 minutes in the lymph vessels and reached the regional lymph nodes. We do not agree, however, with Zhdanov, and we are of the opinion that, essentially, in his experiments too, the propulsion of lymph was caused by the injection pressure. The experiments in question supply accordingly no argument in favour of an active function of the lymphatics.

The effect of the "vis a tergo" seems to be evident in all of the quoted cases. Under normal conditions, however, when there is no question of the transportation of fluids pressed artificially into the lymphatic vessels or the skin, the effect of the "vis a tergo" is rather uncertain and problematic. Movement, be it active or passive, remains, however, in any case a very important factor of lymph flow.

Drinker and Yoffey (1941) attribute the lymph-flow and drainage-promoting effect of movement to a massage of the vessels performed by the adjacent tissues. This applies of course to both active and passive movement. Also other factors are involved, however, in the case of active motions, presumably a change in capillary filtration in the first instance. When moving, the organ is perfused by an increased amount of blood: the capillaries become dilated, filtering surface and filtration pressure increase, and all these factors lead to a more abundant production of lymph. While Drinker and Yoffey found that 4 to 12 mg of lymph flowed from the efferent lymphatics in a minute when the leg of a dog was passively moved, we (Szabó 1954) observed the leakage of 4 mg of lymph per minute (on an average) from a single lymph vessel of the passively-moved dog's leg (it has already been mentioned that Drinker and his collaborators determine the total amount of lymph streaming from an extremity by taking three times the amount collected from a single lymph vessel). Drinker estimates the amount of lymph, discharged by the vessels of the hind leg in walking or running, at 40 to 50 mg/min.

Kubik's experiments (1952) may also be regarded as an important contribution towards elucidating the question of how lymph flow is affected by passive movement. He found that, apart from the ampulla situated at the end of the thoracic duct, and from the Cisterna chyli, there are ampullary dilatations all over the lymph vascular system. Small, spindle-shaped dilatations are, for instance, contained in the afferent lymphatics of the lymph nodes, and he observed similar ampullae in the subserous efferent lymphatics, in the lymphatics of the tongue, etc. Kubik divided these ampullae in two groups. The

to prove the existence of this "vis a tergo" is the observation that, if ligated, efferent lymphatics fill very promptly, become swollen and may even burst as a consequence of lymph congestion. It has been noted that pressure in closed lymphatics may reach a very high value. Intravascular pressure in the lymphatic capillaries under normal conditions, on the other hand, is very low and does not usually exceed 1 to 2 cm water (McMaster 1947). "Vis a tergo" does not, therefore, sufficiently explain the phenomenon in question.

Genersich (1871) and Paschutin (1872) were the first to demonstrate that no lymph, or hardly any, escapes at rest from the lymphatics of the leg. Their finding has since been confirmed by numerous authors (e.g. Asher 1927; Drinker and Field 1933; Rouvière and Valette 1937). This lack of flow does not mean that during the inactivity of the extremity, there exists an equilibrium between capillary filtration and absorption, and consequently no formation of lymph. We have had occasion to point out that even if the extremity is in a state of complete quiescence, the preponderance of filtration alone may lead to the formation of oedema. The preponderance of filtration over reabsorption was amply proved by those experiments in which a steady flow of lymph was produced by passive movement. Haynes (1932a), for instance, induced a slight but uniform flow of lymph in the efferent lymphatics of the dog by a continued passive movement of the leg. We, too, were able to confirm this observation in our own experiments (Szabó 1954; Szabó and Magyar 1955a, 1956). McCarrell (1939a, b; 1940a, b) succeeded likewise in maintaining a continuous lymph stream in the cervical trunk by a steady passive movement of the head.

These experiments could still be explained by an effect of the "vis a tergo". The significance of movement for lymph production was pointed out in the preceding chapter: not only active but also passive movements constitute an important factor in the passage of fluids from the interstitial space into the lymph capillaries.

However, beyond its effect on lymph production, muscular activity is in our view also a very important factor in lymph flow. The earlier results of Japanese investigators fully support this view. Funaoka (1930), as also Funaoka et al. (1930), observed, that radio-opaque matter when injected into a lymph vessel remained immobile for a considerable length of time if the animal was at rest, but was quickly propelled on as soon as an active or passive movement of the organ concerned was provoked. Henry (1933), for example, observed in the course of experiments on the rabbits ear in a transparent chamber that fluids introduced into the small lymphatics streamed at a rapid rate under the effect of even small injection pressures.

This seems to apply also to the experiments of Hudack and McMaster (1933): they injected very small amounts of dissolved dye into the human skin and found that they entered the lymph capillaries and small efferent lymphatics very readily. The rate at which lymph is flowing in the efferent vessels of the skin is comparatively high so

We think that, few as these examples are, they will suffice to demonstrate the significance of movement in lymph flow. The following question remains, however, still open: what is the mechanism which determines the direction of the lymph flow induced by movement? It is in this connection that the valves with which lymphatics are provided play a decisive role. Rouvière and Valette (1937) were justified in pointing out that intralymphatic pressure is continuously increasing from the periphery towards the centre. If, therefore, lymphatics did not contain valves, any lymph flow from the periphery towards the large veins would be inconceivable. Valves are so arranged as to prevent a backflow of lymph. Valves, regulating the direction of lymph flow, are — with the exception of the capillaries — to be found everywhere, even in the smallest efferent lymphatics of animals of the higher orders. A direct observation of rabbit ears enabled Henry (1933) to show that, by means of anastomotic connections, fluid can flow in any direction in the lymph capillaries; however, once it has passed into an efferent lymphatic provided with valves, flow becomes inevitably unidirectional, no matter how small the lymphatic happens to be. Numerous other similar observations are reported in the literature.

Active and passive movement, muscular contraction, intestinal peristalsis, respiratory movement, as also a pressure upon any given area, such as the massage of an extremity or the rabbit ear, have the effect of pressing lymph out of the lymphatics of the area concerned. Valves allow drainage in one direction only so that the lymph is forced to flow towards the thoracic duct, the large collecting lymph channels. It is then in these collecting channels that the "vis a tergo", i.e. the pressure of lymph pressed out of the smaller lymph trunks, may become active. The view that the decisive factor in ensuring the flow of lymph is an active or passive movement of the part of the organ concerned is shared also by Asher (1927).

However, flow in the large efferent lymphatics depends according to our present knowledge on other factors as well. It is known that negative intrathoracic pressure, the thoracic inspiratory suction effect, has a great importance for the inflow of venous blood into the left atrium. Conditions in the large lymph trunks are similar to those observable in large veins. Here, too, there is a thin-walled tubular system filled with fluid in which pressure is low and flow sluggish. External negative pressure acting upon the proximal portion of the tubular system exerts a suction on the fluid in the tubes. Most (1917) pointed out that filling and emptying of the thoracic duct, or rather of its dilated ampullary terminal portion, are correlated with the respiratory movements. In expiration, the ampulla is filled from the direction of the thoracic duct as can be seen from the stream of particles after the injection of India ink as well as from the swelling and blackening of the ampulla. It is, on the other hand, emptied in inspiration. The effect of respiratory movements upon lymph flow has been

first group is formed by the intermuscularly situated ampullae: they are compressed when the muscles contract, while their walls — anchored to the fibres of the connective tissues — are drawn apart when the muscles relax, so that the ampullae play the part of a sort of pressure pumps. The valves allow the fluid pressed out to flow in one direction only, while lymph is sucked in from the periphery when the ampullae become dilated. The other group consists of the ampullae situated in the loose connective tissue and before the lymph nodes, and Kubik suggests that their task is principally the storage of lymph.

In view of these considerations it seems natural that a fairly high amount of lymph flows from the heart, a constantly moving muscular organ. Led by the assumption that the cardiac lymph of dogs is drained by a single lymphatic, Drinker and his associates (1940) inserted a cannula into an efferent cardiac lymph vessel and collected, on an average, 10 to 20 mg of lymph per minute. (Our own experiments [Földi, Romhányi, Rusznyák, Solti, and Szabó 1954a, b] led to the conclusion that, generally, the heart of the dog is drained by two principal efferent lymphatics; we found that ligature of *both* these lymphatics induced interstitial oedema and very marked functional [ECG] alterations in some of our cases).

Motion also constitutes an extremely important factor of lymph flow in other organs. Reference has already been made to the experiment of Drinker et al. which proved respiratory movement to be the most important factor in pulmonary lymph flow. If the thorax is opened and artificial respiration stopped (the oxygen required by the animal being supplied by means of constant insufflation of pure oxygen) lymph will cease to flow in the efferent pulmonary lymphatics, while increased frequency or intensity of the respiratory movements will be followed by increased lymphatic drainage. We do not want to discuss here interventions made with a view to increasing filtration pressure in the lung by a higher intrathoracic negative pressure (e.g. forced breathing or inspiration against some resistance), but we think we must point to the fact that impeded or hindered expiration also promotes lymph flow although it would be expected to diminish the production of lymph (Drinker 1945).

Lymph flow in the intestinal and mesenteric lymphatics may likewise be increased by the peristalsis of the intestines (Drinker and Yoffey 1941); while the motion of intestinal villi has, according to Beznák (1937), no essential effect on the flow of lymph from the intestines. Horstman (1952), explains intestinal lymph drainage by another mechanism. Relying on the evidence of his mostly morphological investigations, Horstman advances the theory that the thin-walled intramural lymphatics run in the intestinal wall between two layers of the musculature (a longitudinal and a circular) so that their contractions exert a considerable influence on the flow of lymph. Such arrangement of the intestinal musculature enables it — according to Horstman — to function as a lymph pump.

from the thoracic duct is quite natural and, strictly speaking, nothing else is proved by Kubik's observations; it is, on the other hand, only to a system of rigid tubes that the hydrodynamic law, according to which the diminution of lateral pressure in a tube exerts a sucking effect upon its side branches, can be applied. In the case of soft-walled and collapsible thoracic duct, one would be justified to expect from an *internal suction not so much an increased lymph flow as rather* a relaxation of the vessel walls, a closure of the lumen or — in other words — a diminishing of the flow.

The mechanism postulated by Kubik cannot, therefore, play a decisive role in the maintenance of lymph flow. The same must be said of Tendeloo's theory (1925) who, describing the factors which influence lymph flow, emphasizes the fact that, in diastole, blood and — together with blood — lymph is sucked in by the heart from the veins. Since the publication of Tendeloo's work it has become common knowledge that no negative pressure which could produce a suction *effect on the veins ever arises in the right atrium; did, however, the* heart exercize such an action, it would surely rather lead to a closure of the collapsible veins.

Blood circulation does nevertheless affect lymph flow beyond doubt. Most (1917), in addition to the fluctuations of flow which are synchronous with respiration, described also another kind of pulsation which can be observed in the thoracic duct during the interval between inspiration *and expiration, a pulsation which has the same* rhythm as the cardiac contractions. It is in our view probable that this rhythm is just a transfer of cardiac or aortic pulsation to the thoracic duct, *i.e. nothing but the consequence of a direct mechanical action. Lee*, for instance, is of the opinion (1923/24) that it is to changes in intrathoracic pressure, provoked by cardiac contractions, that the fluctuations of pressure in the thoracic duct should be attributed. Luciani (1911), on the other hand, was of the opinion that the arteries dilating at each systole, thus transmitted a direct impulse to the perivascular lymphatics for a centripetally-directed discharge of their contents. This theory was shared by Beck (1924). In 1867 Hering (cit. Heller 1869) pointed to the phenomenon that arterial pulsation affected flow in the adjacent lymph vessels. Cressman and Blalock (1939) emphasized that, *situated* between aorta and vertebral column, the Cisterna chyli was in an ideal position to take over aortic pulsation These authors opened the thorax of dogs, tied a cannula into the *Cisterna chyli and connected it to a manometer. The end of the cannula* was placed below the diaphragm so that cardiac activity had no direct effect on the records. This arrangement enabled them to *register pressure fluctuations in the cisterna which were synchronous* with aortic pulsation. A similar pulsation, synchronous with that of the large vessels, was observed when the cannula was tied into the *cervical portion of the thoracic duct or into those large collecting* lymph trunks which pass over or along the aorta in the abdomen.

treated by numerous earlier authors (e.g. Weiss 1861; Colin 1879; Camus 1894; and others).

Respiration, that is the movement of the diaphragm, influences lymph flow also in another way. It was Jossifov (1914, 1930) who called attention to the fact that the distended initial portion of the thoracic duct, i.e. the Cisterna chyli, passes through the diaphragm and lies in the Hiatus aorticus. Therefore, in expiration, the cistern is compressed and the lymph driven out by the diaphragm, while in inspiration the cistern becomes dilated. Jossifov introduced the term "passive lymph heart of mammals" for the cisterna chyli.

Zhdanov (1952) thinks that the lymph-flow-promoting effect of *respiratory movements relies, in the main, upon this* mechanism suggested by Jossifov. He refers to Beck's investigations (1924) who found pressure changes in the thoracic duct to be independent of changes in the negative intrathoracic pressure. Pressure curves show variations of respiration also in curarized animals with open thorax and artificial respiration. However, these experiments do not, in our view, prove that fluctuations of thoracic pressure, the negative intrathoracic pressure, have no effect on lymph flow: what they prove is only that respiration influences lymph flow by mechanisms other than the *pumping effect.*

Kubik (1950, 1952) demonstrated the existence of still another mechanism. He isolated that part of the thoracic duct which joins the large veins and injected India ink into an abdominal lymphatic. The path of the India ink particles can be well observed in the thin-walled duct. Kubik observed the same phenomenon as did Most in 1917: the amount of lymph flowing from the duct into the veins was insignificant during expiration and the ampulla at the end of the thoracic duct become stained with dissolved India ink, while it was emptied during inspiration. He attributed this phenomenon to those pressure changes which occur in the large veins in connection with respiration. In order to test the correctness of this assumption, he isolated the jugular and subclavian veins as also the inferior Vena cava in the body of a dead dog and perfused them with fluid. He found *that when pressure in the veins was raised by preventing the* outflow of the fluid, all flow from the thoracic duct to the veins came to an almost complete standstill and that a sudden drop of intravenous pressure, produced by the removal of the obstacle, provoked a marked acceleration of the outflow of the fluid containing India ink particles from the duct. Kubik concludes that the decisive factor in the maintenance of lymph flow is neither the thoracic pressure nor the consequent negative pressure in the large veins but a sudden drop of pressure in them. It increases the rate of venous flow, causes a considerable reduction of lateral pressure which exerts a pumping effect on the thoracic duct.

We are of the opinion that Kubik's experiments cannot be accepted as conclusive evidence. That a rise of venous pressure impedes flow

branches coming from the vagus and the intercostal nerves. According to Wriesberg, innervation of the Cisterna chyli is supplied in the main by the XIth thoracic ganglion and the left splanchnic nerve. Innervation of the thoracic duct and the main lymph trunks was studied more recently chiefly by Russian investigators (Timofeyev 1897; Dogiel 1897; Kitmanov 1901; Lawrentjew 1925/26; etc.).

Lawrentjew found a periadventitial nerve plexus around the thoracic duct of dogs which contained also small ganglion cells. Fibres running *to this plexus arise from the collateral trunk, which is parallel to the* sympathetic trunk, and from the intercostal nerves. Fibres coming from Kondratew's Ganglion supremum lead to the upper cervical *portion of the thoracic duct,* while thin rami *arising from the greater* splanchnic nerve lead to the lower portion.

In contradiction to Lawrentjew who worked with methylene blue, Zhdanov and Pavlitskaya (1949) investigated the intramural nerve plexus of the thoracic duct with the method of silver impregnation. Their experiments essentially confirmed Lawrentjew's findings.

*Not only the thoracic duct but the other efferent lymphatic channels*, too, receive their innervation from the sympathetic and parasympathetic system. Lawrentjew (1927a, b), for example, studied the innervation of the lymphatics in the large intestine, Kositsyn (1953) that of the lymph nodes, Kubik (1954) that of the mesenteric lymph vessels, etc.

The presence of muscle fibres in the wall of lymphatics as also the autonomic innervation of the lymph channels indicate that, under the regulating influence of the nervous system, lymphatics are capable of active functions, e.g. contraction with a consequent change of *calibre or closure, etc. Activities of this kind cannot fail to affect* lymph flow.

Earlier experiments performed by us (Rusznyák, Földi and Szabó 1949, 1950) had the object of studying the effect which stimulations of the sympathetic trunk produce on lymph flow.

Experimenting with dogs, we isolated the sympathetic trunk on the left side between the aorta and the Psoas major, and placed a well-insulated electrode on the nerve. The sympathetic was then stimulated by means of faradic current, while a radio-opaque substance (30% ioduron or 35% perabrodil) was subcutaneously introduced into both hind legs.

As could be seen from a series of rontgenograms, the radio-opaque matter filled the lymphatics of the posterior extremity on the unstimulated *side generally within 1 to 3 minutes and advanced as far* as the lymph nodes of the bend of the knee, while no filling of the lymphatics was observable on the stimulated side (Figs. 164 and 165). We attributed this phenomenon to a contraction, a spasm of the lymphatics elicited by the irritation of the sympathetic trunk. Only the left, i.e. the stimulated, side seemed to be affected : the lymphatics of the right side filled quite normally.

It is, however, not merely in the thoracic duct and the large lymph trunks that lymph flow is influenced by arterial pulsation. To prove the correctness of this statement was the object of experiments performed by Clark and Clark (1933), Henry (1933) McMaster and Parsons (1938) as also by Parsons and McMaster (1938b).

Making their experiments in "transparent chamber" on the ear of rabbits, Clark and Clark found that, while advancing at a slow rate, lymph in the lymphatics moved to and fro in synchronism with arterial pulsation. Henry (1933) is quite justified in remarking that the definitely unphysiological conditions under which the experiments were performed hardly allow these observations to be accepted as conclusive.

Nor do the experiments of Parsons and McMaster (1938a) seem to be fully convincing. Perfusing isolated rabbit ears with defibrinated blood at a constant pressure, these authors found that hardly any lymph flowed from the ear and that it became oedematous after some time; if, however, a pulsating blood flow was maintained in the ear by means of a pump, lymph flow become considerably more vigorous and no oedema developed.

The above-described experiments allow in any case the conclusion that cardiac activity, arterial pulsation, affects lymph flow and promotes it quite as much as the other factors discussed in the foregoing paragraphs such as respiratory movements, intestinal peristalsis, contraction of the intestinal muscles, muscular contractions in general, active and passive movement of the extremities, massage etc.

All these factors are, however, hardly sufficient to explain the flow of lymph from certain parenchymatous organs. Let us take the liver as an example: in comparison with the weight of this organ hepatic lymph flow is very abundant and uniform although there is no question of either active or passive movement and in spite of the fact that the effect of arterial pulsation must be relatively slight as the liver receives most of its blood supply from the portal system. What, *then, is the mechanism which maintains lymph flow* in such cases?

## INNERVATION AND TONUS OF LYMPHATICS

It has long been known that the wall of certain lymphatics contains [illegible] content [illegible] t organs [illegible] a single [illegible] 52; etc.). In addition nerve fibres run to the wall of the lymph vessels. Lymphatics have a sympathetic and a parasympathetic innervation.

Autonomic nerve fibres running to the Cisterna chyli and the thoracic duct were described by Wriesberg (1780) and Cruikshank(1789, 1790). According to Cruikshank, the thoracic duct is innervated by

branches coming from the vagus and the intercostal nerves. According to Wriesberg, innervation of the Cisterna chyli is supplied in the main by the XIth thoracic ganglion and the left splanchnic nerve. Innervation of the thoracic duct and the main lymph trunks was studied more recently chiefly by Russian investigators (Timofeyev 1897; *Dogiel* 1897; *Kitmanov* 1901; *Lawrentjew* 1925/26; *etc.*).

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It was demonstrated in the further course of our experiments that the effect of stimulation could be stopped by the denervation of the femoral artery or the infiltration of its adventitia with novocain. The effect of stimulation remains unchanged if the sciatic nerve is



*Fig. 161.* Lymphatics filled with radio-opaque substance (right leg) after the injection of 5 ml of 30% ioduron into the paw

sectionized, while the section of the femoral nerve weakens the effect to a slight extent so that a thin and pale strip of the radio-opaque substance remains still visible in spite of faradization. It seems, therefore, that most of the lymphomotor nerve fibres of the lower extremity run in the adventitia of the femoral artery and some of them perhaps in the femoral nerve.

Bellinazo and Monteverde (1953) repeated our experiments and came, on the whole, to results similar to ours. They observed a constriction of the lymphatics immediately after the section of the lumbar sympathetic, as also after the stimulation of the sciatic nerve or as



*Fig. 165.* Hardly any radio-opaque substance visible in the lymphatics of the left leg of the same dog after the stimulation of the left symphatetic trunk

an effect produced by artificial thrombophlebitis. If, however, symphatectomy had been performed 8 days before the experiment, they saw a dilatation of the lymph vessels.

Led by the results of our experiments we, too, have recommended periarterial novocain infiltration around the femoral artery as a

treatment of thrombophlebitis in human patients, a matter to which we shall have occasion to refer in the later course of this work. Such treatment yielded satisfactory results and we observed a considerable diminution of thrombophlebitic oedemas in the lower extremities.

The effect produced by neural stimulation upon the peripheral lymphatics was studied also by Rouvière and Valette (1937). Their experiments gave negative results. These authors declare that nervous stimuli have no effect on lymph flow. They applied faradic current to the superficial nerve fibres of dogs but failed to register any progression of the dye-labelled lymph in the lymphatics. However, if the electrodes were placed directly around the lymph vessels, or on the *surrounding connective tissue, stimulation elicited movement* of the lymph. It is suggested by these authors that this effect is by no means due to neural stimulus but the result of a mechanical action which the electrodes exert upon the thin-walled lymphatics. We think Zhdanov (1952) was right in having criticized this one-sided "mechanical" concept.

It was by a stimulation of the ischial nerve that Rouvière and Valette accelerated the flow of lymph in the efferent lymphatics of the limb. This old and rather uncertain observation originates from Lewachew (1886); it furnishes, however, no better argument in favour of the effect of neural stimuli on lymphatics than do numerous other *similar observations which may be better explained* by a change of haemodynamic conditions and, consequently, of lymph production. This may be the reason why pertinent data reveal so many contradictions. Rogovitz (1885), for instance, observed increased lymph flow after having cut the sciatic nerve, while Paschutin, before him (1872), had seen no such increase.

Bert and Laffont (1882) made their experiments by a direct observation of the lymphatics. According to their report, a constriction of the lacteals can be induced by the stimulation of the mesenteric *nerve fibres, and a dilatation by stimulation of the splanchnic nerve.* They found these alterations to be independent of the condition of the blood vessels so that the effect in question was not due to a change in haemodynamic conditions.

While the literature is poor in reports on the effect of neural stimuli upon the peripheral lymphatics, it contains numerous such publications concerning [illegible] trunks. Some of these publicatic [illegible] g.

It was claimed by C [illegible] r-striction of the thoracic duct could be induced by stimulation of the sympathetic trunk, while stimulation of the distal end of the left splanchnic nerve caused the Cisterna chyli to contract. The existence [illegible] seems to be proved by those experi- [illegible] of atropine was followed by a relaxa- tion, [illegible] asm, *of the wall of the thoracic duct.*

Valeyeva (1948) studied the effect of different drugs on the isolated thoracic duct. Its tone was increased by adrenaline and weakened by atropine, nicotine and quinine. The thoracic duct was perfused with various drugs in another group of her experiments. Adrenaline, barium chloride and physostigmine provoked marked constriction, caffeine and quinine induced dilatation.

Studying the efferent lymphatics in the testicle of rats and guinea pigs, Pullinger and Florey (1935) likewise found that they contracted under the influence of adrenaline and pituitrin. Lymphatics in the ear of mice gave no such response.

But the tonus of lymph vessels is governed not only by stimuli arising from the autonomic nerves. It seems that the activity of the lymphatics is also regulated by the central nervous system. It was demonstrated by Camus and Gley as long ago as 1895 that stimuli applied to the sensory nerves might elicit a contraction of the thoracic duct. It is claimed by Kohanina (1948, 1949a, b, 1951) that lymph flow is influenced also by stimuli applied to the pressoreceptors and chemoceptors of the intestinal canal, kidney, renal pelvis and spleen. Neither these experiments nor those of Camus and Gley (1895a, c), in which they found that lymph flow was accelerated by asphyxia, are in our view very convincing: they do not prove that the effects observed were due to a direct action of the nervous system on the lymphatics. Kokhanina's experiments were performed under decidedly unphysiological conditions (perfusion of an isolated intestinal loop or of the renal and splenic vessels with Tyrode solution); besides, the intervention probably affected rather the production of lymph than the vasomotion of lymphatics. Nor do we find those experiments of Kokhanina more convincing in which she demonstrated that lymph flow was influenced by pain reflexes. A stimulation of the proximal end of the divided sciatic nerve with induced current increased the lymph flow of naturally respiring dogs to 6·0 to 18 times its original rate, while no such acceleration could be provoked by the same stimulus in animals with artificial respiration. The acceleration of lymph flow under the influence of pain was attributed not only to a change in the respiratory movements but, in the first instance, to increased blood pressure (?). We are of the opinion that — while, in addition to respiratory and haemodynamic changes, a direct effect of the stimulus (transmitted through the lymphomotor centres of the central nervous system) upon lymphatic vasomotion may play a certain role in Kokhanina's experiments — the mechanism postulated by her surely needs additional experimental corroboration.

It was further demonstrated by Kokhanina that locally or generally applied heat, too, gives a stimulus which affects lymph flow. She suggests that this is due to a reflex which, arising from the receptors of the skin, affects the contractile elements of the lymphatics by way of the spinal cord. We would refer in this connection to the first part of this work in which it was pointed out that, presumably, heat

stimuli induce a local increase in lymph production. Kokhanina performed also other experiments with a view to determining the effect of reflexes induced in the carotid sinus: however, her experimental method was also in these experiments of such a nature as to make it probable that the stimuli affected the production rather than the flow of lymph.

The results of Kokhanina's experiments are, therefore, somewhat ambiguous. We think the effects observed by her are complex inasmuch as heat stimuli or stimulations of the carotid sinus affect the tonus of capillaries and, consequently, capillary pressure, and may at the same time increase capillary permeability so that lymph production becomes more vigorous. Simultaneously, perhaps also the reflex mechanism postulated by Kokhanina may become operative.

Uryupov (1954) studied the effect produced by the stimulation of the receptors of internal organs upon biliary and lymph flow. He found that (in 21 instances out of a total of 27) increased pressure in the urinary bladder, the kidney and the superior mesenteric artery led to increased lymph flow in the thoracic duct. A decrease in pressure went hand in hand with a decrease in lymph flow. Since, meanwhile, blood pressure underwent no change, the author concluded that the phenomenon could not have been due to a change in haemodynamic conditions. The reflexes in question appeared even after a division of the spinal cord (at the height of segments D III—IV) and the extirpation of the vagus so that the reflex must have been peripheral but not an axonreflex. Our criticisms of Kokhanina's experiments, i. e. that her experimental conditions were such as to leave it undecided whether a change in the amount of lymph flowing from the thoracic duct was brought about by a change in lymph production or in lymph flow (lymphatic tonus), are also to some extent applicable to Uryupov's findings.

Objections raised in connection with these experiments were taken into account by Kovanov (1952) who employed a more appropriate method in his investigations into the mutual reflexes between lymph vascular and blood vascular system. The method, first used by Valeyeva (1948) in the Physiological Institute of the Bashkir Medical University, consists, essentially, in the following: a cannula is inserted into the thoracic duct below in the thoracic cavity and the cannula is perfused with a fluid. Tying another cannula into the upper end of the duct in the neck, the fluid flowing through it is collected. With this arrangement, Kovanov was able to ascertain that, by clamping the common carotid artery, the amount of fluid flowing through the thoracic duct invariably diminished by about 10 to 50 per cent in spite of constant infusion-pressure, while there occurred a simultaneous rise in blood pressure. Electrical stimulation of the carotid sinus leads, on the other hand, to a considerable dilatation of the thoracic duct and the amount of outpouring fluid increases two to fourfold. Thus, these interventions provoke a simultaneous dilatation and contraction of the blood and lymph vessels.

Intravenous injection of adrenaline was found by Kovanov to increase the amount of fluid flowing from the thoracic duct although a perfusion of the duct itself with adrenaline narrowed its lumen decidedly. It is supposed by the author that the intravenous administration of adrenaline releases a reflex, the result of which is an increase in the capacity of the lymphatic system so that it can take up a greater amount of capillary filtrate. A stimulation of the splanchnic nerve, too, is followed by an effect similar to that of adrenaline. Our own experiments (Szabó and Magyar 1956) failed to yield similar results. After intravenous adrenaline injections we observed rather a constriction of the thoracic duct.

Neither the arguments nor the conclusions of Kovanov should be accepted uncritically. We find, for instance, the following passage in his report: "Rise of blood pressure after obstruction of the carotid is due neither to a constriction of the blood vessels nor to the lymph being driven out of the lymphatics. Contraction of the lymphatic vessels diminishes the filtration of blood plasma into the lymph vascular system." Remembering all that has been written in this work regarding capillary filtration and lymph production it seems unnecessary to point out in detail the errors of this concept.

A recent report of Petrovski (1954) describes the work performed in the Physiological Institute of the Bashkir Medical University with a view to elucidating the role played by the lymph vascular system in the regulation of blood circulation. Valeyeva's above-discussed method (isolated perfusion of the thoracic duct or the Cisterna chyli) was used in the experiments. Kovanov observed a dilatation of the thoracic duct consequent upon a constriction of the portal vein and the inferior vena cava. He concluded that pressoreceptors were contained also in the wall of veins, the stimulation of which dilated the thoracic duct. It should be added that — as is visible from the published graphs — a ligature of the vena cava and the portal vein causes a fall of arterial pressure which, as had previously been demonstrated by Kovanov (1952), gives rise to a dilatation of the thoracic duct. The experiments, described in the said report, do not, therefore, prove beyond doubt that there are receptors in the wall of veins.

By tying off the pulmonary vein or injecting a 4% gelatin solution into the pulmonary artery, Smirnov (1955) produced a rise of pressure in the pulmonary artery and found that the thoracic duct and the cervical trunk became temporarily dilated also in this case. Here, too, the dilatation of the lymphatics was accompanied by a simultaneous sharp fall of peripheral blood pressure. Therefore, to prove that lymphatic dilatation was due to a reflex coming from the pulmonary artery it is necessary also in this case to exclude the possibility of any role played by a fall of pressure in the systemic circulation or a reflex arising in the carotid sinus. Smirnov's observation that the reflex-induced dilatation of the lymphatics continues even after the section of the vagus when a constriction of the pulmonary

vein provokes no, or hardly any, drop of blood pressure, seems to confirm Petrovski's hypothesis. It should be noted, however, that lymphatic dilatation in these conditions is considerably less pronounced.

Worthy of note are the experiments concerning the effect of adrenaline on the thoracic duct. We have already discussed Kovanov's experiments (1952) in which the intravenous injection of adrenaline caused a dilatation of the perfused thoracic duct and increased the amount of outflowing fluid, while the perfusion of the thoracic duct itself with adrenaline elicited its contraction. A few years before, Valeyeva (1918), too, had made the same observation. It was finally Petrovski (1954) who arrived at the conclusion that intravenous injections of adrenaline induced sometimes a dilatation and sometimes a constriction of the thoracic duct. There can be no question of a direct effect of adrenaline even in cases where the intravenous injection *of the drug is followed by a constriction of the thoracic duct.* Very high doses of adrenaline have to be added to the perfusate if even a moderate contraction of the duct is wanted, while even quite small doses elicit its spasm if the drug is administered through the intravenous route. This phenomenon leads Petrovski to the conclusion that the constrictor action of adrenaline is of reflex origin. He regards this assumption as corroborated by the observation that intravenous injections of physiological saline may have an adrenaline-like effect on the lymph vascular system. The pressor reflexes arise from the veins. Petrovski refers in this connection to experiments in which it was demonstrated that a narrowing of the inferior vena cava led to a distension of the lymphatics, while a narrowing of the superior vena cava led in numerous instances to a contraction of the thoracic duct. A contraction of the duct was further observed when the walls of the superior Vena cava had been distended by the introduction and inflation of a rubber balloon. Finally, a contraction of the thoracic duct occurred also after the injection of a small volume (20 to 40 ml) of Locke's solution into the superior Vena cava.

Adrenaline, introduced into the circulation, may thus — according to Petrovski — elicit a contraction of the thoracic duct by way of reflex action, an action determined by the effect of adrenaline on blood pressure and/or on particular portions of the vascular system. *That the calibre of the thoracic duct and other lymphatic* channels is influenced by such reflexes points to the probability that the tonus of the lymphatics is regulated centrally and that this regulation is simultaneous with that of the blood vessels.

We agree with these considerations and cannot but regret that Petrovski and his collaborators did not proceed to a full elucidation of the mechanism which regulates the mutual action of reflexes between the lymph vascular and the blood vascular system. Reflex actions regulating blood and lymph vessels are, according to Petrovski, moving in the same direction: increased tonus of the blood vessels

goes hand in hand with an increased tonus of lymph vessels, while a paralyzation of the vasomotor centres (e.g. by hexane or pentothal) means the reduction of blood pressure and the simultaneous dilatation [illegible] and lymphomotor centres as identical and claims that the centre of the lymphatic vessels forms part of the vasomotor apparatus.

It is further suggested by Petrovski that changes in the tonus of lymphatics play an important part in the regulation of blood pressure and in the maintenance of normal blood circulation. When, for instance, blood pressure falls after acute loss of blood, not only the depots of blood but also the "lymph depots" become empty and a passage of fluid from the lymphatics into the blood stream occurs which tends to restore the volume of circulating blood. This theory is debatable, and we should prefer to say that the lymph vascular system plays a certain role in the mobilization of the extravascular pool of protein. We cannot, however, accept Petrovski's concept that, after loss of blood, lymph (the total amount of which he estimates at about 6 litres — following in this respect Iwanow) pours so copiously into the blood vascular system as to become a decisive factor in maintaining blood pressure. After a loss of blood, interstitial fluid flows — as is known — directly into the circulatory system and the amount of fluid conveyed by the thoracic duct can surely not suffice to restore circulating plasma to its original volume. Nor can we quite understand why it is supposed by Petrovski that a fall of blood pressure following loss of blood induces a *contraction* of the lymphatics and a consequent discharge of lymph into the blood vessels; it was he, himself, who observed a dilatation of all examined lymphatics after a fall of blood pressure produced in his experiments: this would mean rather a lowering of the tonus of the lymphatics so that — in theory at least — more lymph would escape from the dilated lymph trunks, in contradiction to what is stated by Petrovski elsewhere, namely that "they retain the liquid part of the blood".

In addition, Petrovski assumes that the vasomotor centre is paralyzed in shock which leads to a dilatation of the lymph and blood vessels and a passage of a part of the blood plasma into the distended lymphatics. The results of our own experiments contradict this assumption.

While, according to the above, we are unable to accept many of Petrovski's conclusions, we regard some of the experiments performed at his institute as extremely important. We attach especial significance to his conclusion that haemodynamic disturbances may provoke changes not merely in the tonus of blood vessels but in that of lymphatics as well and that, on the other hand, changes in the tonus of lymphatics may give rise to changes in that of blood vessels. In this connection we would refer to Valeyeva's investigations (1948, 1949) who demonstrated that a rise of pressure in the perfused thoracic

duct went hand in hand with a rise of arterial pressure, a finding confirmed by Kubik (1953, personal communication). Tchernigovski's and Burtchik's experiments (cit. Kovanov 1952), in which it was shown that *reflexes influencing blood circulation and respiration* can be elicited by a stimulation of the receptors of the lymph nodes, admit of a similar interpretation.

Recently it was stated by Valeyeva (1954) that not only the thoracic duct but also the Cisterna chyli contained pressoreceptors. She made experiments in which the lower and upper ends of the Cisterna chyli were isolated in the abdominal and thoracic cavity of anaesthetized dogs and both sides cannulated; the isolated cisterna was then perfused with physiological saline under different pressures (20 to 80 mm Hg). Increase of perfusion pressure induced a decrease of blood pressure in the carotid artery by about 12 to 15 mm Hg. A rise in the pressure of perfusion, on the other hand, was followed by an average drop of 12 to 15 mm Hg in the blood pressure. This points to the presence of pressoreceptors in the Cisterna chyli whose stimulation decreases blood pressure.

The experimental results quoted in the foregoing paragraphs allow the *conclusion that lymphatics possess a vasomotor* innervation, stimulation of which elicits contraction, spasm, of both the thoracic duct and the peripheral efferent lymphatics.

But innervation has not the sole task of provoking a spasm of lymphatic vessels under the effect of certain stimuli: it serves also in the maintenance of their physiological tonus. That this is so seems to be confirmed by the experiments of Prives (1918): he found that, after the subcutaneous injection of radio-opaque material, many more lymphatics were filled in dead than in living dogs, and that the X-ray pictures of the lymphatics filled with the radio-opaque substance were much sharper. This observation might also be interpreted by the assumption that lymph-capillary permeability becomes higher in the dead body (Rusznyák, Földi and Szabó 1954) and so facilitates the entry of the substance into the lumen of the lymphatics. Another explanation would be that part of the lymphatics is closed in the living individual or that the lumen of the lymph vessels is narrower on account of their physiological tonus.

The problem of the physiological tonus of lymphatics has been neglected. Even Zhdanov's monograph (1952) mentions it only in passing. The only report bearing on the subject we have been able to discover apart from Kovanov's above-discussed experiments is that of Goldstein (1950) in which it is stated that there exist collateral reserve lymph paths which come into action whenever the drainage of lymph through the lymph trunk encounters difficulties and that — a finding of importance from our point of view — an opening of these collaterals occurs also after partial sympathectomy.

Seeing that these data were not enough to decide the question whether lymphatics really have a physiological tonus and if lymph

flow can be accelerated by a reduction of the tonus, we deemed it necessary to make further experiments with a view to clearing up the problem.

Since the reported data seemed to prove that the physiological [illegible] amine (4-4 dibenzyl-β-chloroaethylamine), a drug which is known to abolish the effect of both adrenaline and noradrenaline was used for blocking of the sympathetic. In addition, we examined in some cases also the effect of a ganglion-blocking substance (vegolisen) on the flow of lymph.

After anaesthetizing dogs with chloralose, we isolated their thoracic duct and tied a polyethylene cannula into it. Lymph was collected at intervals of 10 minutes and dibenamine was then slowly infused into the animals intravenously. 1 mg of the drug, dissolved in 1 ml of physiological saline, was administered *per minute*. This dose was chosen to avoid, as far as possible, any significant fall in blood pressure, the value of which we measured by a mercury manometer tied into the femoral artery.

Dibenamine had the effect of increasing the flow of lymph in all our experiments. Such increase was sometimes preceded by a temporary reduction of lymph flow. The action of the drug on lymph flow is shown diagramatically in Figs. 166 and 167.

The graphs make it clear that increased lymph flow is independent of a fall in blood pressure (Fig. 166).

Similar results were obtained with the use of hexamethonium, although they were less pronounced and uniform, a phenomenon quite natural in view of the fact that hexamethonium paralyzes not only the sympathetic nerve endings but the synapses as well.

The effect of chlorpromazine (largactil) on the lymph flow in dogs was recently examined in our Institute by Papp and Stark (1957b). Out of a total of 9 cases, lymph flow diminished (at the rate of 15 to 50 per cent) in seven, remained unchanged in one, and increased (by 15 per cent) in one case. A significant fall in arterial mean pressure occurred simultaneously. It is by no means easy to evaluate the results: largactil not only affects blood pressure, cardiac output, venous pressure, capillary pressure and permeability but exerts also a sympatho-adrenolytic and ganglion-blocking, even a direct vasodilator effect. Such complexity of the mechanism makes it rather difficult to precisely determine that factor which is responsible for the diminution of lymph flow as observed by the authors.

Besides the increase in lymph flow produced by dibenamine, another characteristic change was that observed in the composition of the lymph: its protein concentration became markedly lower. While increased lymph flow became apparent soon after the administration of dibenamine, the fall in protein level only manifested itself after some time.

It should be noted that the mechanism through which dibenamine exerts its action upon the flow and composition of the lymph is a complex one.

It was demonstrated by Fejfar and Brod (1951) that venous pressure diminished *under the effect of dibenamine. It could, therefore,* be supposed that increased lymph flow is a secondary phenomenon provoked by a reduction of pressure in the large veins. Such suppo-

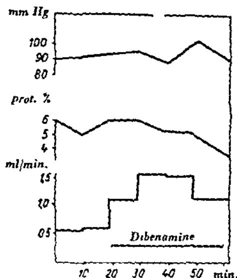



*Fig. 166.* Effect of dibenamine on blood *pressure, lymph flow and the protein* level of the lymph

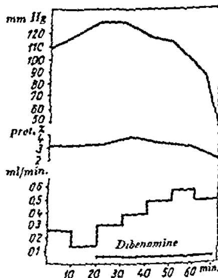



*Fig. 167.* Effect of dibenamine on *blood pressure, lymph flow,* and the *protein level of the lymph*

*sition would be wrong for two reasons. One the one hand,* dibenamine exerts no significant effect upon normal venous pressure and affects only a pathologically increased pressure; on the other hand, there was in our experiments no connection between thoracic duct and veins.

*Transitory fall in arterial blood pressure,* due to decreased peripheral resistance, is the most characteristic of the haemodynamic changes induced by dibenamine (Brod and Fejfar 1951). It is, therefore, possible that — as a consequence of diminished arteriolar tonus — *the gradient between precapillaries and capillaries becomes less steep; a diminution of the arterial blood pressure is thus accompanied by* an increase in capillary pressure which may, of course, give rise to increased transudation and lymph formation. Although the results *of our experiments do not entitle us to reject this possibility, we are* inclined to regard it as improbable, for increased lymph production on the periphery need not necessarily entail increased flow of lymph from the thoracic duct. It should be noted that, in our experiments,

administration of dibenamine was followed by increased lymph flow irrespective of whether blood pressure fell or rose, i. e. whether haemodynamic changes occurred or not. These facts do not, therefore, prove that the action of dibenamine is based on an increase in the rate of lymph production.

We think the effect of dibenamine is due to the action by which this sympatho-adrenolytic agent diminishes the normal physiological tonus of the lymphatics which, thus, become dilated. The effect of dibenamine is, therefore, essentially similar to that observed in our earlier experiments after the resection of the lumbar sympathetic trunk.

*As mentioned a justified objection which might be raised to our* experiments would be that dibenamine does not affect the tonus of the lymphatics but induces changes in the production of lymph through altered haemodynamic conditions. To counter such objection we performed additional experiments so as to further substantiate our theory.

After anaesthetizing some dogs with evipan and others with chloralose, we isolated one of the lymphatics running along the saphenous vein and introduced into it [illegible]
used ir [illegible]
as alsc [illegible]
infusec [illegible]
isolate [illegible]
minut [illegible]

These experiments showed that fluid infused into the peripheral lymphatic did not quantitatively get into the thoracic duct (Fig. 168). Lymph flow in the duct not only failed to increase adequately but expressly decreased in some of the cases.

In another group of experiments, performed also on dogs, the lumbar sympathetic trunk on the left side was resected and physiological saline infused on the same side into a lymphatic which ran along the great saphenous vein in the hind leg. As in the preceding experiments, lymph was collected through a cannula tied into the thoracic duct.

After sympathectomy, the amount of lymph flowing from the thoracic duct was found to have considerably increased during the infusion.

Still another group of experiments (Szabó and Magyar 1957) had the object of studying the direct effect of dibenamine on the tonus of the thoracic duct.

Dogs of both sexes with a body weight of 8 to 12 kg were used. A method, similar to that employed by Valeyeva (1948) for the first time, was elaborated for the perfusion of the thoracic duct. Under evipan anaesthesia, the abdomen was opened and the Cysterna chyli or rather the abdominal portion of the thoracic duct isolated. A polyethylene cannula was then introduced and pushed up to the height of the diaphragm. This done, another cannula was inserted into

that point of the thoracic duct in the neck where it empties into the veins. Where the thoracic duct emptied into the large cervical veins through a number of branches, all discoverable small lateral branches were ligated. Fluid — at a constant pressure of 15 to 20 mm $H_2O$ — was then infused into the thoracic duct through a cannula applied to its distal end. We determined the

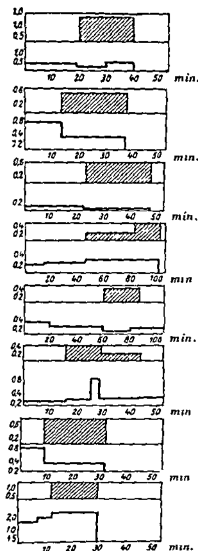


Fig. 168. Lymph flow in the thoracic duct during intralymphatic infusion of fluid (normal dogs)

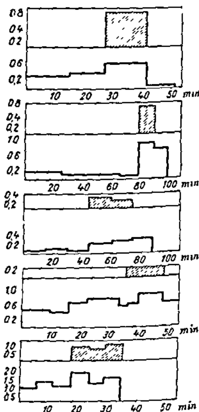


Fig 169. Lymph flow in the thoracic duct during intralymphatic infusion of fluid (sympathectomized dogs)

Hached injected fluid ml/min ; white: lymph flow in thoracic duct ml/min.

amount of inflowing fluid by means of a bubble flowmeter, and collected at the same time the fluid outflowing from the cervical portion of the duct. The perfusate consisted of physiological saline to which 10 mg % Evans-blue had been added as indicator. In some of the cases also 5% dextran was added. A mercury manometer tied into the femoral artery was employed for the determination of blood pressure. The dose of intravenously administered dibenamine was 10 mg/kg which — after 3 to 4 preliminary periods of 10 minutes — we infused slowly, during approximately 10 minutes. In some cases not dibenamine but adrenaline was infused, likewise slowly and through the intravenous route; the total dose was from 1 to 5 mg according to the reaction of the blood pressure.

A total of 20 such perfusion experiments gave the result that the amount of fluid which flowed into the thoracic duct at a constant pressure of about 20 cm $H_2O$ varied from case to case. Allowing a free flow in the system composed of the pressure vessel itself, the bubble flowmeter and the connecting rubber or polyethylene tubes, the amount of outpouring fluid was slightly above 10 ml/minute, while it dropped to an average of 2·75 (0·2—7·4, s.d. = 2·17) ml/minute after the thoracic duct had been connected to the system (i.e. after the introduction of the polyethylene cannula into the abdominal portion of the duct). This decrease was obviously due to the resistance of the thoracic duct which depends in the first instance on the diameter of the duct and — though probably to a much lesser extent — also on its length, i. e. on the distance between the cannula attached to the abdominal portion of the duct and that tied into its cervical portion. As regards the lumen of the lymph trunk, probably we have to deal not only with anatomical differences between individual animals but also with differences in the tonus of the lymphatic.

Led by such considerations, we divided our results in two groups according to the volume of fluid flow. In 7 cases, where the amount of inflowing fluid had a mean value of 0·65 ml per minute (0·22—1·28, s.d. = 0·255) an increased tonus of the thoracic duct was supposed, while 13 cases with a mean inflow rate of 4·0 ml per minute (2·2—7·4, s.d. = 1·78) constituted the second group. We determined the limiting value between the two groups at 2 ml per minute (for both in and outflow). We supposed that — if our concept was correct, i.e. if one had to deal in these cases not only with anatomical but also with functional differences affecting the rate of flow — the dilating, i.e. the flow-promoting effect of dibenamine was bound to manifest itself much more markedly in cases with a slow initial rate of flow.

The volume of fluid pouring from the cervical portion of the thoracic duct was approximately identical with what has been infused into its abdominal end: it was, on an average, 0·62 ml per minute (0·08—1·70, s.d. = 0·615) in the 7 cases of the first category; 4·1 ml

per minute (2·2—8·0, s.d. = 1·77) in the second category, and 2·83 ml per minute (s.d. = 2·25) as the mean of both groups.

That the volume of infused fluid was approximately the same as that collected at the other end of the thoracic duct argues against the possibility that only one — perhaps the smaller — of the lateral branches at the cervical end of the thoracic duct was open in our experiments, while the other branch was allowed to drain freely into the venous system. Had this been the case, the volume of introduced fluid ought to have been significantly greater than that of

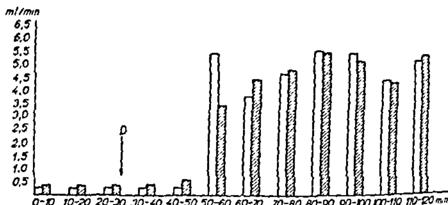



*Fig 170.* Effect of dibenamine on the tone of the thoracic duct

White columns outflow of fluid from the duct, (ml/min.), hachured columns inflow of fluid to the duct, D 10 mg/kg of dibenamine i.v

the outflow. True, while the respective mean values of inflow and outflow were approximately identical, there were fairly considerable differences between inflow and outflow in individual cases: it is nevertheless safe to say that a methodical error of this nature, one that could have falsified our general results, did not impair our experiments.

We performed 17 experiments to study the action of dibenamine. In 15 cases, the administration of the drug was followed by an augmentation of lymph flow (i.e. inflow of fluid from the infusion system and its outflow through the open cervical end of the thoracic duct), while no such augmentation was observed in 2 cases. As expected, augmentation was much more pronounced in cases with a lower rate of initial flow. Both of the negative experiments were cases with a relatively great initial flow. Therefore, in some cases where the initial flow was very great, we increased the tonus of the thoracic duct by means of adrenaline before examining the effect of dibenamine.

Figs 170 and 171 illustrate, each, a typical case. Fig. 170 shows one in which the rate of initial flow was low, while in Fig. 171 a

case is presented in which flow was rapid even before the administration of dibenamine. As a rule, augmentation of flow went hand in hand with a fall in blood pressure; however, in 3 cases the fall in blood pressure was considerably preceded by the augmentation of lymph flow, i.e. a reduction of the tonus of the thoracic duct. The reason that we attach importance to this observation is that a fall in blood pressure leads, according to Petrovski (1954), by way of reflex action to a decrease in the tonus of the thoracic duct so that it would be conceivable that augmentation of flow was not a direct effect of dibenamine in our experiments but may have arisen as a secondary

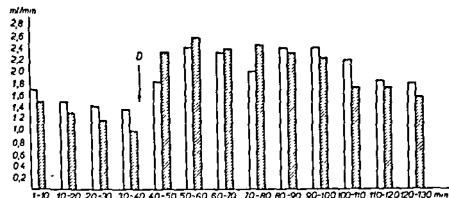



*Fig. 171.* Effect of dibenamine on the tone of the thoracic duct
Notations as under Fig 170

phenomenon by a stimulation of the pressoreceptors contained in the circulatory system (carotid sinus).

Increase in flow varied from case to case. Taking the average value of the preliminary periods as 100 per cent, the mean value of inflow after the administration of dibenamine rose to 337 per cent, with a mean value of 721 per cent (152—2500) in the seven cases of the first and one of 139 per cent (98—200) in the ten cases of the second group. Even if the case with the outstandingly high percentage of 2500 in the first group is disregarded, the mean value of inflow in this group still rose to 308 per cent after the administration of dibenamine against 207 per cent, the mean value of inflow in the total of 16 cases (the total also being considered omitting the exceptional case). These figures seem to furnish a sufficient proof of the fact that flow in the thoracic duct is significantly augmented by the action of dibenamine and that, on the other hand, dibenamine elicits a considerably more pronounced decrease in the tonus of the thoracic duct if the initial tonus is high. As regards the flow-promoting effect of dibenamine, the difference between the two

groups (set up according to the volume of *initial flow*) is *very significant* also statistically (with Wilcoxon's method, $p \leq 0{\cdot}1\%$).

We also examined the effect of adrenaline on lymph flow in five instances where initial flow was rapid and, in accordance with our theory, the tonus of the thoracic duct low.

It was found that adrenaline decreased in- and outflow in every case. This effect manifested itself, however, only during the infusion of the drug and was of a very short duration. The average rate of inflow in the examined 5 cases was 4·8 ml per minute (3·1—7·4, s.d. = 1·82), that of the outflow 4·6 ml per minute (2·4—8·0, s.d. = = 2·18) before the administration of adrenaline and decreased, on an average, by 21 (8—34) and 27 (4—57) per cent, respectively, under the effect of the drug. Minimum values after the administration: inflow, 3·9 ml per minute (2·0—6·2, s.d. = 1·76); outflow, 3·5 ml per minute (1·9—6·5, s.d. = 2·35).

Decrease in flow was, thus,both temporary and small. The difference between pre-adrenaline and post-adrenaline values was statistically not significant ($p > 40\%$). This is to say that, although a slight decrease of flow was invariably observable during adrenaline infusions, it cannot be said that a parenteral administration of adrenaline exerts a marked tonus-promoting effect on the thoracic duct. It is, of course, possible that a greater number of experiments might furnish a better proof of this effect since, as has been said, a slight decrease in flow was demonstrable in every one of our cases.

To sum up, on the results of the described experiments *we have reached the following conclusions.*

Dibenamine, a drug of sympatho-adrenolytic action, markedly lowers the tonus of the thoracic duct. The degree of this effect depends on the tonus of the lymphatic duct before the administration of the drug: if the initial tonus of the duct is low (i.e. initial flow is rapid) the effect of dibenamine is considerably less than in cases with slow initial flow. This effect is presumably due to a direct paralysis of the adrenergic innervation of the thoracic duct rather than to a reflex provoked by a fall in blood pressure. Our results have thus confirmed our earlier assumption that sympathectomy and dibenamine affect lymph flow not only by inducing a decrease in precapillary tonus, an increase in capillary pressure and the consequent augmentation of lymph production but also by causing a decrease in the tonus *of the thoracic duct and the other lymphatic vessels. Recent* investigations of Drozdova (1953) confirm this concept. She examined the collateral lymph circulation in the sympathectomized and deafferented intestinal loops and found its lymphatics considerably dilated in comparison with normal conditions. Increased number of anastomoses between the mesenteric lymphatics were observed. Such phenomena remain visible for as long as 1 to 2 months after sympathectomy, while after the lapse of 2 to 3 months there remains hardly any difference that would distinguish the picture from that

of the normal lymph vascular system. Partial sympathectomy leads to a dilatation of the lymphatics and the loss of their physiological tonus, if even for a short time only.

It has been demonstrated in the foregoing paragraphs that, possessing a characteristic physiological tonus, lymphatics are capable of independent contraction and relaxation. This function stands under the regulating influence of the nervous system. By these variations of their tonus, the lymphatics affect lymph flow in an active manner, and, by way of reflex spasms, are able to prevent the drainage of lymph or interstitial fluid from a given area which may then give rise to fluid retention, oedema and, in certain circumstances, grave morphological and functional alterations.

Beyond this, however, lymphatics take an active part in the transportation of the lymph and in the maintenance of lymph flow. Without such active function it would be difficult to understand how lymph can flow in areas where there are hardly any external forces (movement, respiration, arterial pulsation, etc.) to promote lymph flow.

## ACTIVE MOTION OF THE LYMPHATICS

As long ago as 1869 Heller gave evidence for the fact that the mesenteric lymph vessels of guinea pigs perform about ten rhythmical contractions per minute. The number of contractions diminishes premortally, after anaesthesia of long duration, but the mesenteric lymphatics continue to contract 3 times a minute during about an hour even after the death of the animal. The lumen of the lymphatics greatly distends during contractions. Contractions occur in a segmental sequence, i.e. those of the peripheral precede the contractions of the central segment. Lymph flow through the corresponding valves begins before the systole of the peripheral segment, simultaneously with the diastole of the central segment, but the flow is strongly accelerated by the systole of the peripheral section.

These lymphatic pulsations are completely independent of either arterial pulsation or respiratory movements. Heller declared that lymphatics exerted a pumping effect throughout their length, but raised the question whether such rhythmical lymphatic contractions occurred also elsewhere in the animal organism and if they were common to all mammals. This problem arose because Müller and Schwann (cit. Heller 1869) had failed to observe similar contractions of the mesenteric lymphatics of rabbits.

This question has remained a battleground of conflicting theories till our own days, i.e. during almost a whole century. Basing his evidence on a single negative observation, Florey (1927a) denied the existence of lymphatic pulsation in mans. He made this negative observation during an operation: prior to laparotomy, he gave his patient oil and, pulling the mesentery forward, examined the

lymphatics filled with chyle. He found them dilated but could not observe contractions.

However, we must agree with Horstmann's criticism (1952) that one should be extremely cautious in jumping to conclusions of this kind. It is stated by Horstmann, who — like Heller — observed rhythmic contractions in the mesenteric lymphatics of guinea pigs, rats and also pigs, that pulsation may cease after laparotomy for 10 to 15 minutes and, not infrequently, fail altogether to come into action again.

Florey (1927a), too, found rhythmically pulsating lymphatics in guinea pigs. He estimated their frequency at about 8 to 10 contractions per minute and declared that their rate rose in certain instances to 22. Pulsation may sometimes cease for a duration of 10 to 15 seconds and stops altogether after death, but the lymphatics continue to fill with chyle as long as the intestines remain active. According to Florey (1927a), the lymphatics of the diaphragm also display a rhythmic pulsation in the guinea pig. Pfuhl and Wiegand (1940), on the other hand, described a lymph-heart-like structure in the omentum of the same species.

Webb (1933) examined and photographed contractions of the mesenteric lymphatics in the rat. Florey described them as movements of a peristaltic character, but Webb's description seems to contradict this finding. Lymphatic contraction formed the subject of a number of reports, e. g. those of Sappey (1874/1885), Lieben (1910), etc.

Clark and Clark (1932), as also Henry (1933) described irregular, apparently spontaneous, lymphatic contractions in the ear of rabbits.

In contradiction to the earlier observations of Florey (1927a) it was subsequently found by Pullinger and Florey (1935) that the thoracic duct of the guinea pig performs rhythmic contractions perfectly similar to those of the mesenteric lymphatics. In guinea pigs and rats, rhythmic contractions were further observed in the efferent lymph trunks of the thigh and testis. Administration of adrenaline was followed by a spastic contraction of these channels Using a method described by them, these authors examined the lymphatics in the ear of mice also, but failed to observe pulsation.

Horstmann (1952), employing histological methods, investigated the mesenteric lymphatics of guinea pigs and humans, and studied also the function of these vessels in the guinea pig and the rat. Histological investigations (freshly fixed material) led him to the conclusion that the aspect of the medium-sized lymphatics was extremely different from valve to valve. He regards each valvular segment (i. e. the length between two valves) as a separate functional unit. Differences in the histological structure of the particular segments (thickness of wall, position of muscle layer, etc.) express the essential functional state of these portions. Morphological examinations makes evident that, structurally, there is no difference between the lymph

vessels of man and guinea pig. He found in both of them the same difference between different valvular segments as regards cross section and wall-thickness of the lymph vessels. A comparison of the results of his morphological and functional investigations convinced Horstmann that human mesenteric lymphatics must perform the same rhythmic contractions as had earlier been described in guinea pigs.

Horstmann regards lymph vessels not as continuous tubes but as systems composed of serial tubules. The musculature of each segment, i.e. each tubule, of the mesenteric lymphatics becomes gradually weaker towards the valves. The contraction of the valvular segment (i.e. the tubule between two successive valves) propels the lymph to the next segment: when the segment is filled, its muscles contract and force its lymph contents to move in a proximal direction. The only possible functional interpretation of the morphological pictures in man and guinea pig is the assumption that the lymphatics undergo rhythmic contractions and that these contractions effect the propulsion of lymph. Differences in the thickness of the musculature of the different segments cannot be understood unless it is assumed that one has to deal with different states of contraction. The segmental transport, visible in histological pictures, cannot be explained either by a "vis a tergo" or a "vis a fronte" (e.g. intrathoracic pumping action). Forces propelling lymph forward cannot arise but in the lymphatic vessels themselves.

Observations made on the mesentery of living guinea pigs point to the fact that lymphatic contractions belong to a type of fairly complicated movements. That lymph flow is discontinuous follows from the contraction and relaxation of the valvular segments: their contraction is, however, not simple but a complex process consisting of repeated rhythmic contractions of one or more segments. Each wave of lymph propulsion requires, therefore, several contractions.

All observations quoted above point to the fact that lymphatics play an active role in lymph transport. This function is performed by rhythmic contractions of the lymph-vessel walls which propel the lymph. That the direction of the propulsion is proximal is due to the arrangement of the valves. A function of this nature has been proved in respect of the mesenteric lymphatics of guinea pigs and rats, and seems to be highly probable in man. Rhythmic contractions have furthermore been observed in the thoracic duct and the efferent lymphatics of the thigh and testis of guinea pigs and in the ear of the rabbit, etc.

Recently, Jancsó (1954) studied the structure and functional significance of the lymphatic valvular apparatus. He observed structural differences in the different segments, of subcutaneous lymphatics and in their valves. Following the course of a single lymphatic in the neighbourhood of the inguinal nodes of the mouse, he encountered valves without ampullar expansion of the lymph vessel, further

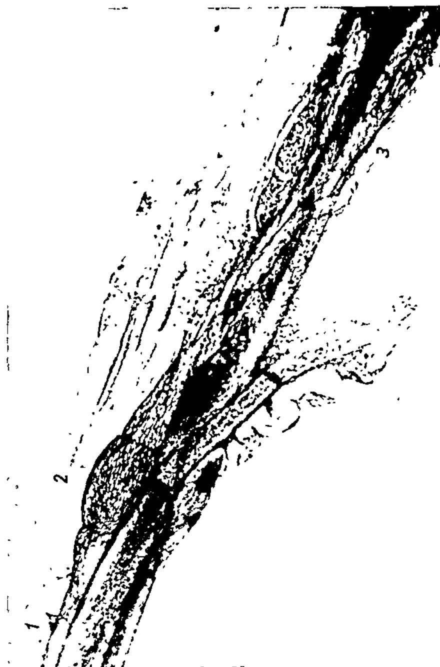



*Fig 172.* Lymphatic close to the inguinal lymph nodes (mouse). Conspicuous difference between the structure of the valve segments (Jancsó 1955)

1 = valve without ampullar expansion of the vessel (see Fig 173), 2 = regular spindle-shaped ampullar structure (bulbus), 3 = retort-shaped but not double-valvular structure (see Fig. 174)



*Fig. 173.* Enlargement of Fig. 172, valve without ampullar expansion (Jossé 1955)



*Fig. 174.* Retort-shaped but not double-valvular structure (Jancsó 1955)

*regular spindle-shaped ampullar structures with a double valvular* arrangement, and finally a retort-shaped but not double valvular apparatus. Jancsó thinks it quite possible that there are lymph-heart-like, lymph-propelling apparatuses in the subcutaneous lymphatics also. The double valves seen point decidedly to a function of this nature, *and the presence of two closely applied valves with* an ampullar distension of the vessel can be best explained by the assumption that a segment with such structure acts as a pumping apparatus.

In summing up the above considerations we can safely say that lymphatics — *at least in certain species and in certain areas* — promote lymph *propulsion* and help to maintain lymph flow by their active contractions. Apart from such physiological function, a change in the tonus of the lymph-vessel walls may occur in certain circumstances: lymphatics undergo spastic contraction which may give rise to a blockage and a consequent *insufficiency of lymph flow.*

## SPASM OF LYMPHATICS IN INFLAMMATION

The evidence of our own investigations (Földi, Romhányi, Rusznyák *and* Szabó 1950) *seems to suggest that* lymphatic spasms play a prominent role *in the formation* of inflammatory oedema.

Oedema is known to be a typical concomitant symptom of inflammations and appears, independently of aetiology, in all inflammatory processes. It is generally accepted that oedema is caused by *a disturbance of equilibrium between the forces which are responsible* for capillary filtration and those responsible for the reabsorption of the filtrate into the vascular path. There is, besides, another important factor that plays a role in this connection, namely, a disturbance in the removal of interstitial fluid by the lymphatics. In creased filtration *in inflammatory processes is surely a factor that must not* be disregarded: the permeability of capillaries is increased too, and protein, even erythrocytes, escape from the blood stream and gain access to the interstitial space. Only the lymph vessels are capable of *removing* protein and corpuscular *elements which have escaped* into the tissues. But an increase in the amount of exudate with a high protein level in the area of inflammation is possible only if the lymphatics are unable to carry off the fluid which, owing to their increased permeability, has escaped through the capillary walls. This may be due *to two reasons. First,* it is possible that the lymphatics are unimpaired and yet unable to cope with their augmented task so that we are faced with a dynamic insufficiency of the lymphatic system, a possibility substantiated by Heidenhain's observation that the *lymphatics coming from* the *inflammatory area contain much fluid* with a high protein level. Second, Menkin's (1931a, b) experiments, discussed elsewhere in this book, justify the assumption that lymphatics in the inflammatory area are obstructed by fibrinous coagulum.

It was demonstrated by Menkin that neither colloids nor corpuscular particles were reabsorbed from inflamed tissues which induced him to speak of a "fixation" in the inflammatory area. On the other hand, he, too, observed an increase in capillary permeability: dye and bacteria, introduced by the intravenous route, accumulated at the site of inflammation.

Contradictions in the literature and the importance of the problem induced us to study the anatomical and functiona alterations of lymph flow in connection with inflammatory oedema.

In the first group of experiments (Földi, Romhányi, Rusznyák and Szabó 1950) we revised Menkin's statement that substances introduced into the inflammatory area are not absorbed. It has been noted that lymphatics can be filled by the subcutaneous injection of radio-opaque material (ioduron, per-abrodil) and that their course can thus be studied on X-ray films. We provoked inflammatory oedema by various methods in our *experiments: 1 to* 2 ml of turpentine oil, or the same amount of staphylococcus toxin, or a 40% dextrose solution was subcutaneously injected into the paw of the hind leg of dogs. The paw became swollen, red and oedematous on the next day. We then injected 10 ml of 30% ioduron into the inflammatory area and roentgenographed it. No shadows of lymph vessels could be observed in any of the films made in this group of experiments.

Such negative result must have been due either to the fact that, for some reason, the radio-opaque substance failed to gain access to the lymphatics, or else to the fact that the injected substance had become so diluted by the exudate of the inflammatory area that, though it penetrated into the lymphatics, it was too diluted to be visible on the X-ray film. With a view to deciding this question we infiltrated the hind leg of a normal dog with physiological saline to such an extent that its volume became larger than that of the inflamed paw. This done, we injected 10 ml of 30% ioduron into the infiltrated area. While, in this case, the X-ray films showed filled lymphatics, they were much paler than usual. Therefore, we repeated these control experiments with 70% ioduron and obtained sharp shadows of the lymphatics.

Conversely, in no case did we succeed in filling the lymphatics when 70% ioduron was injected into the inflammatory area. This experiment justifies the conclusion that the radio-opaque substance had really failed to penetrate into the lymph vessels.

Our further experiments had the object of ascertaining the condition of the lymphatics situated cranially from the centre of inflammation. We injected 70% ioduron a few centimetres proximally from the oedematous area. The lymphatics failed to be filled also in this case, an apparant indication of the fact that lymphatics become obstructed not only in the area of inflammation but in a cranial direction as well.

We, therefore, proceeded to examine conditions of absorption ...roximally from the area of inflammation.

Sodium thiocyanate (NaSCN) was used in these experiments, and the method we ...mployed was the same as we have described in detail elsewhere in this work. After subcutaneously injecting 10 ml of the normal solution of the compound into that ...art of the body which was to be examined, we determined the NaSCN-level of the

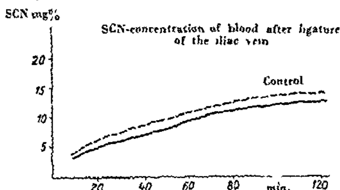



*Fig. 175.* Absorption in venous congestion

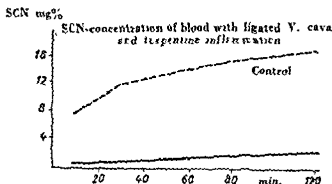



*Fig. 176.* Absorption in venous congestion + inflammation

plasma every ten minutes for two hours. The le[illegible] standard measure of absorption. S[illegible] through blood vessels and lymphat[illegible] or the *inferior V. cava* immediately [illegible] a few days *after the* determination of normal NaSCN-a[illegible] This done, we repeated the NaSCN-*absorption* experiment and found that the rate of absorption had but slightly diminished after the total or partial blockage of the extremity's venous circulation (Fig. 175).

Our procedure in the next group of experiments was, therefore, this: a few days *after* having *ascertained* the *normal absorption* of NaSCN, we provoked an inflammation by means of the above-described method; next day, we tied up the homolateral iliac vein

or the inferior v. cava and examined the absorption of NaSCN anew. It can be seen from Fig. 176 that — under such conditions — sodium thiocyanate, injected by the subcutaneous route cranially from the inflammatory area, is absorbed very ineffectively, a phenomenon which points to the probability that, due to the inflammation, lymphatics become obstructed also cranially from the inflammatory centre.

We then proceeded to a histological examination of the lymphatic obstructions observed in these cases. We found that the lymphatics were, at several points, totally or partially closed by fibrinous coagulum in the centre of inflammation, in the area of leucocytic infiltration and the inflammatory oedema. Numerous dilated lymph capillaries and small lymphatics, which contained serous, oedematous fluid and showed sharp contours, could be observed at the edges of the inflammatory reaction. Collapsed empty larger lymphatics were predominant around the efferent vessels and nerve bundles where, after the injection of India ink, a profuse meshwork of India ink-filled efferent lymphatics is seen under normal (non-inflammatory) conditions. No lymph capillaries could be seen in this aera. In some cases also spastically contracted larger lymphatic paths were observed proximally from the inflammatory area (Figs. 177 and 178). Pathohistological conditions encountered in the inflammation are schematically illustrated in Fig. 179.

As has already been noted, a spastic contraction of the lymphatics can be induced if the lumbar sympathetic trunk is stimulated by faradization. It has also been shown that it is possible to stop this lymphangiospasm by novocain infiltration or a resection of the periadventitial nerve plexus of the femoral artery. In our experiments we tried, therefore, to resolve the spasm of inflammatory origin. Having failed to fill the lymphatics by means of novocain infiltration we resected the lumbar sympathetic trunk on the inflamed side in subsequent experiments. In this manner we succeeded in getting filled lymph vessels in three out of five cases. This result is a further indication of the role lymphangiospasm plays in inflammatory oedema.

It seems to be evident from our experiments that lymphatics are closed in inflammatory oedema. Lymphatic obstruction in the centre of the inflammation is due to the formation of fibrinous coagulum: this constitutes a mechanical occlusion and occurs only in the central part of the inflammatory area. It has, however, been proved that a lymphatic closure, i.e. spasm, of functional character occurs proximally from the centre. The existence of this spasm was proved by the histological observations picture and also by the fact that it was possible to fill the lymphatics with radio-opaque substance after sympathectomy. That, in some instances, we failed to achieve this result in no way invalidates our concept: we think that, in processes of inflammation, anatomical and functional alterations lymphatics of the

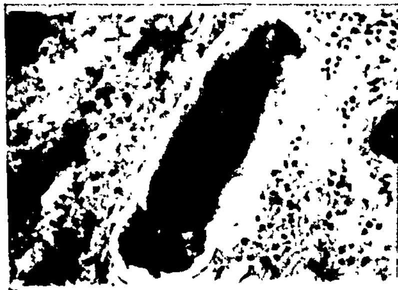


*Fig 177.* Fluid-filled lymphatic in the area of inflammation

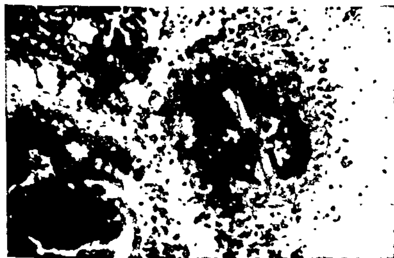


*Fig. 178.* Spastic lymph vessel proximally from inflammatory area

act simultaneously, and it is quite possible that in the given case anatomical occlusion gains ascendancy over functional spasm. (Also, the inefficacy of denervation may be due to that lymphatic spasm occurring in in flammation is elicited partly by local chemical factors or axon reflexes.) The same phenomena can be observed also in cases of thrombophlebitic oedema: both reported data and our own observ-

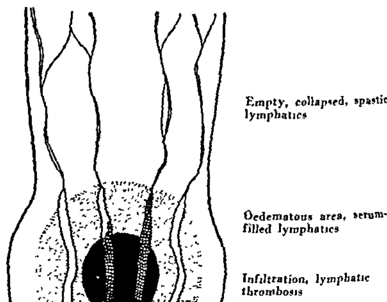



*Fig. 179.* Condition of lymphatics around the inflammatory area (schematic drawing)

ations show that oedema of this kind can be made to *disappear in most cases by* sympathetic blockade.

Leriche and Kunlin (1934) were the first to recommend the novocain infiltration of the lumbar sympathetic trunk for the treatment of thrombophlebitis in the lower extremities. They suggested that, in cases of thrombophlebitis, arteriospasm was provoked by a reflex arising from the wall of the veins which — by way of local anoxia — increased capillary permeability. It was demonstrated by Ochsner and de Bakey (1940, 1949) that a venoarterial reflex of this kind did actually exist and that the amplitude of the oscillometrically measurable arterial pulsations is diminished in the extremity *affected by thrombophlebitis. Experiments in which it was shown* that arterial pulsation is one of the most important motors of lymph flow have led these authors to the conviction that, essentially, the cause of the insufficiency of lymph flow a decrease of due to diminished *arterial pulsation.*

We cannot share this view and, led by the evidence of our own experimental results, prefer a different explanation. It was on account of these results that we began to make perivascular novocain infiltrations in the therapy of patients suffering from thrombophlebitis. Results were, on the whole, highly statisfactory: the swelling of the extremities decreased considerably, and sometimes disappeared altogether, after 2 to 3 days. Stimulated by us, Littmann and Rubányi, too, applied this method and obtained similar results.

Relying on the evidence of our results we are, therefore, of the view that in thrombophlebitis oedema is not caused by local anoxia — which is anyway not certainly proved to increase capillary permeability except in extreme cases — but is rather due to a local insufficiency of lymph flow. Such insufficiency is, however, not induced by a decrease in arterial pulsation since the latter is far from being the most decisive factor of lymph flow in the leg, a muscular organ of active locomotion. Reflex spasm of the lymphatics, by causing a mechanical insufficiency of lymph flow, i.e. one of a functional character, is the decisive factor.

A case, described by Homans (1940), admits of a similar interpretation. One of his patients had a lymphoedema of traumatic origin: a log had fallen on his leg which gave rise to an oedema that resisted all kinds of treatment, and disappeared only after sympathectomy. We think that this, too, was a case of lymphoedema of lymphangiospastic origin. According to Drozdova's investigations (1953), it is, however, possible that sympathectomy reduced the tonus of the collateral lymphatic paths and promoted their dilatation so that lymphoedema may have been due also in the given case to a traumatic closure of the collaterals.

Our experiments enable us to explain the apparent contradiction between the observation that there are lymphatics in the inflammatory area closed by coagulum and Heidenhain's observation who found increased lymph flow in the vessels coming from the said area. Our histological investigations made it evident that there exists an area of lymphatics filled with protein-rich fluid between the central part of the inflammation where thrombosis of the lymphatics is found and that cranially situated portion where a spastic contraction of the lymph vessels is encountered.

It became further evident that not only corpuscular elements injected into the inflamed tissues but also crystalloids become "fixed" in the inflammatory area and the cranially situated adjacent parts. It seems to be clear that a closure of the lymphatics constitutes one of the principal factors in the formation of inflammatory oedema and that it prevents bacteria and other toxic substances from passing from the site of inflammation into the blood stream. Lymphatic spasm is, thus, a kind of practical defence mechanism which — like all such mechanisms — may, however, become harmful under certain conditions. If, for instance, lymphatic closure lasts too long, the

oedema fluid may become organized, fresh connective tissue fibres may form with the result that we are faced with the classic picture of the chronic irreversible lymphoedema. Therefore, in every case when lymphatic spasm does not seem to serve any useful purpose (e.g. thrombophlebitis), lymphatic closure ought to be relieved and the opening of the collateral lymph paths attempted, either by means of blockade or by the resection of the corresponding sympathetic innervation.

## CORRELATION BETWEEN LYMPH FORMATION AND LYMPH FLOW; LYMPHATICOVENOUS ANASTOMOSES

From what has been exposed in the foregoing it seems evident that, in certain circumstances, conditions may arise in the lymph vascular system under which the flow of lymph encounters difficulties. A spasm of the lymphatics may, for instance, prevent the propulsion of lymph, or — again — the lumen of the lymphatic channels may become obstructed as to impede drainage.

If, relying on the evidence of reports in the literature and our own observations, we compare the total amount of lymph flowing in the efferent lymph vessels of different vascular regions with that pouring out from the thoracic duct a certain incongruity becomes obvious. By adding up all the lymph obtained from the various efferent lymphatics we come to a value that is considerably higher than that of the lymph collected during the same time from the thoracic duct.

Data of this kind must, of course, be assessed very carefully, since — as regards magnitude of lymph flow — there exist wide deviations from one animal to the other and even in the same animal at different times. Yet, even the most cautious appraisal of the experimental results cannot exclude the possibility that not all the amount of peripheral lymph is drained by the thoracic duct. The right lymphatic trunk is deliberately disregarded in these considerations, as it is does not bear upon our problem, one which has, nevertheless, also arisen in connection with this lymph vessel.

We will content ourselves with quoting but a few data pertinent to the problem. In the rat, Bollman et al. (1948) collected in the course of 24 hours 5 ml of lymph from the liver, 20 ml from the gastrointestinal tract, and 25 ml from the thoracic duct. Quite apart from the fact that it is hardly possible to make a quantitative collection of intestinal and hepatic lymph, whereas lymph flowing from the thoracic duct is easily measurable, one does not understand what becomes of the lymph of the intrathoracic organs, the kidney, the musculature, the subcutaneous connective tissues, etc.

Although, unfortunately, we failed to find such data as would have enabled us to total the lymph flow of all essential regions in respect of any particular species, we have tried to summarize those reported data which refer to the dog (Table 49).

TABLE 49

| Organ examined | Authors | Lymph flow ml/min. |
|---|---|---|
| Liver | Cain et al. 1947 | 0·225 |
| Heart | Drinker et al. 1940 | 0·015 |
| Hind legs | Drinker 1938 | 0·070 |
| Kidney | Földi and Szabó 1952 (cit. Földi 1952) | 0·50 |
| Intestinal tract | Szabó and Magyar 1956 | 0·065 |
| Total | | 0·88 |
| Thoracic duct | Heidenhain 1891<br>Cain et al. 1947<br>Földi, Rusznyák and Szabó 1952 | 0·40—0·50 |

It is evident that the amount of lymph collected from the thoracic duct per unit of time is considerably less than that produced by all the organs enumerated in the table. Further, our table is far from being complete since it does not include the lymph flowing in a number of organs and regions which, in the dog, surely account for quite a considerable part of thoracic duct lymph. It was proved by our previously described experiments (Földi, Rusznyák and Szabó 1950), too, that not all the fluid introduced into a peripheral lymph vessel gained access to the thoracic duct. We are referring to those experiments in which it was shown that a comparatively large amount of fluid infused into a peripheral lymphatic failed to increase the rate of lymph flow in the thoracic duct.

And so the question arises: what happens to the fluid introduced into the peripheral lymphatics? Several alternatives seem to be possible:

1. The infused fluid passes directly into the blood stream by way of lymphaticovenous anastomoses.
2. The fluid is absorbed into the blood capillaries in the lymph nodes.
3. The fluid passes through the walls of the efferent lymphatics into the interstitial tissue to be absorbed there by the blood capillaries.
4. The fluid accumulates in the lymphatics and the lymph nodes.

Lymphaticovenous anastomoses constituted a baffling problem for investigators during centuries. Anatomists were particularly intrigued by the question why the lymphatics of mammals emptied into the large veins precisely in the lower part of the neck while — except in very rare instances — there exists, as a rule, no connection between lymph vascular and blood vascular system.

The first investigators of the lymphatic system, e.g. Stenonis (1662), Nuck (1692), Meckel (1772) and others were of the opinion that the lymphatics of the extremities and the parenchymatous organs were directly connected with the veins of the corresponding areas. This concept arose from the fact that mercury, the only substance available in those times for the injection of lymphatics, disrupted the vessel wall and so gained access to the veins. This error was pointed out even by contemporary critics of these authors, e.g by Hal[illegible] 1952).
moses [illegible] of the anatomy of the lymphatic system (Bartels 1909; Jossifov 1930; Rouvière 1932; Sushko 1950; Zhdanov 1952) are, however, almost without exception of the view that — save where the main lymph trunks empty their contents into the cervical veins — there exists, as a rule, no communication between the lymphatic and circulatory system either in man or, generally, in the mammal.

This notwithstanding, even up-to-date literature contains reports that run counter to this concept. It was, for example, declared by Baum (1911) that the lymphatics of dogs and bovines emptied in certain cases into the sacral vein, jugular vein, V. circumflexa femoris prof. and the cephalic vein. Job (1918) demonstrated lymphaticovenous anastomoses in the abdomen and thorax of rats; these communications appeared irregularly and had a very narrow calibre which made their accurate investigation technically impracticable. Another theory of long duration was that the lymphatics of the thyroid were drained directly by the veins (Caylor and co-workers 1927), a hypothesis which was not borne out by the results of our own investigations (Földi, Jellinek, Rusznyák and Szabó 1954). The theory in question seems to be invalidated by the fact that, in our experiments, a ligature of the main cervical lymphatic trunk resulted nearly always in a dilatation of the lymphatics in the tyroid and a lymphoedema of the gland. It is obvious that the ligature of the main cervical lymph collector could not have provoked lymphatic congestion if a direct communication between the lymphatics of the thyroid and the venous system had existed.

While it is held by most authors that certain developmental anomalies may be accompanied by the formation of anomalous connections between lymphatics and veins (Wutzer 1834; Arnold 1845, cit. Zhdanov 1952; Pic, Anson and Burnett 1944; etc.), Frautschi (1938, 1948) claims that lymphaticovenous anastomoses are of regular occurrence also in humans. Not less than 35 lymphaticovenous anastomoses were observed by Frautschi in 25 cases out of a total of 50 autopsies of human corpses. It is claimed by this author that a perfection of the dissecting technique enabled him to encounter communications between veins and lymphatics in nine out of his last ten cases of autopsy. He concludes that such communications exist in all cases,

without exception. He encountered anastomoses at the height of the XIth thoracic and the IInd lumbar vertebra: they were mostly connected with the system of the V. cava.

In his criticism of Frautschi's investigations, Zhdanov (1952) remarks that it is obvious from the published drawings that one is dealing here with artefacts. Zhdanov examined more than 100 corpses and failed to encounter such anastomoses in any of them. Sushko (1950), who examined the lymphatics of the lung, liver, stomach, pancreas, appendix, uterus, ovary and thyroid on a very large material in the Anatomical Institute of Kiev, arrived to the same conclusion. Sushko emphasizes the difficulties encountered by investigators who want to examine intraorganic lymphatics with the method of indirect injection, i.e. by introducing dye-stuffs into the parenchyma of the organ concerned. It is very easy in such cases for the injected substance to penetrate into the veins which, however, is no proof in favour of the existence of lymphaticovenous anastomoses. Never in the course of his investigations (performed in injected preparations) did he succeed in demonstrating a direct communication between lymphatics and veins, neither in respect of the intraorganic lymph vessels nor in that of the extraorganic efferent lymphatic trunks. A similarly negative result was reached by Gryaznova (1955): after tying off the azygous vein and closing the intercostal veins, she injected a dye-stuff into the thoracic duct which failed to pass into the veins even at a high injection pressure.

It is known that if we tie off the thoracic duct of the dog, the lymph will find access to the large veins in some of the cases: this process takes place in a short time, according to some authors within a few hours (Drinker and Yoffey 1941). Naito (1932, cit. Zhdanov 1952) affirms that a ligature of the thoracic duct and the right lymphatic duct invariably leads to the death of the animal and that no newly-formed connections between venous and lymphatic system can be found. However, this observation stands isolated in modern literature and disagrees with the experiences of Kishi (1935), Blalock and co-workers (1937), as also with our own observations. It was reported by Rodriguez, Carvalho and Pereira (1933), too, that communication between thoracic duct and large veins was re-established in two out of ten dogs which had survived the grave operation of having the large lymphatics tied off.

In addition to these investigations of a morphological nature we posses many data of a functional character which prove that the artificially interrupted communication between the main lymph trunks and the veins is promptly restored.

Biedl and Decastello (1901) demonstrated that the number of lymphocytes circulating in the blood diminishes rapidly after the ligature of the thoracic and the right lymphatic duct. This is presumably because the cells, formed in the lymph nodes, pass — under normal conditions — into the circulation through the lymphatic

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aticovenous anastomoses. Congo red, bound to protein was used. The dye-protein complex is only slightly diffusible, and it is fairly improbable that it would be absorbed through the blood capillaries at a higher rate even if it could pass through the wall of the lymph capillaries. In none of our experiments was it possible to find Congo red in the blood which shows that, at best, only a negligible fraction of the amount of intralymphatically infused dye could have passed into the blood circulation.

Let us take a concrete example. In our experiment No. 4, 82 mg of Congo red, contained in 16·5 ml of fluid, was infused into a peripheral lymphatic of a dog of 12 kg body weight. Supposing the volume of the animal's circulating plasma to be 0·5 l, one would have expected to find a 16 mg % concentration of the dye in the plasma if the total amount of dye had passed into the blood circulation. But even if only 5 per cent of the infused dyestuff had passed through lymphaticovenous anastomoses into the blood path, the level of Congo red in the plasma should still have amounted to about 1 mg %, an easily demonstrable concentration. We have already stated that not even traces of the dye were detected in the plasma.

These results are in contradiction to those of Glenn and co-workers (1949): they introduced the dye T-1824 (dissolved in plasma) into a lymphatic of the leg and it appeared (in 4 out of 5 cases) after some 15 minutes in the blood plasma even in dogs with thoracic-duct fistula. This convinced the authors of the existence of functioning lymphaticovenous anastomoses. We think it is rather doubtful whether — in the given concentration (1 per cent solution) — the whole quantity of the dye is actually adsorbed to the plasma proteins. If some of the dye remains unadsorbed, it may diffuse from the lymphatics and may become reabsorbed by the blood capillaries. It is, of course, also possible that an absorption of the dye occurred in the lymph nodes. It should moreover be noted that the authors omitted to give information about the quantity of dye they had found in the blood plasma so that it is not clear whether a significant percentage of the infused substance found access to the circulation.

All these considerations confirm our opinion that, though the existence of lymphaticovenous anastomoses may be demonstrated in anatomical preparations, they are functionally negligible under normal conditions. This applies not merely to those collateral lymphatic paths which — described by some and denied by other workers — are directly drained by the abdominal and thoracic veins but is valid also in respect of lymphaticovenous anastomoses postulated in the lymph nodes. Therefore, under normal conditions, any considerable quantity of lymph, as such, can pass into the blood path solely in the lower part of the neck where the large lymph trunks join the venous system.

We do not mean to say that fluids and dissolved molecules cannot get from the lymphatics into the blood capillaries. We have already

trunks. The number of lymphocytes per ml drops, according to these authors, from 2147 to 1146 six hours after the blockage of the large lymphatics; there were only 452 lymphocytes in the blood after 24 hours; the number was almost unchanged (494) after 48 hours, and went up again to 1523 after the lapse of four days. Similar results were reported also by Davis and Carlson (1909/1910), Bunting and Huston (1921), and Lee (1922).

These results are easily explained by the assumption that lymphocytes gain access to the blood circulation through newly-formed lymphatic paths a few days after the ligation of the normal lymph channels.

In contradistinction to this assumption, Zhdanov (1952) thinks it possible that — in given circumstances, i.e. when lymphatic drainage is hindered for some reason — it is not through newly-formed collateral paths that lymph is drained but that lymph, aided by a sharp rise of pressure in the sinusoids, may find access to the veins within the lymph nodes. He refers to Schulze's experiments (1925) who detected stomata in the endothelial wall of the postcapillary veins of lymph nodes: these stomata serve as communications between blood vessels and lymph paths in the cortical substance of the lymph nodes

Kubik's investigations (1952) are also highly noteworthy. He thinks that the histological structure of the lymph nodes argues in favour of fluid absorption. Arteries emptying into lymph nodes are conspicuously wide and form an extremely delicate and very profuse network of capillaries in the area of the marginal sinuses: each follicle is surrounded by a dense capillary reticulum. The vessels have a stellate arrangement and are suggestive of the angioarchitecture as seen in the cortical substance of the kidney. The capillaries are drained by exceptionally wide veins (cf. "sinus principle" of Kiss 1954b). The number of afferent lymphatics entering the lymph nodes is, moreover, always in excess of that of efferent lymph vessels leaving the nodes, and the sum of the cross sections of afferent vessels is considerably larger than that of the efferent lymphatics. All these facts furnish fairly convincing proofs for the existence of fluid absorption in the lymph nodes (Fig. 180).

The evidence of our own investigations argues against the existence of such direct connections between lymphatic and venous system as would be able to ensure lymphatic drainage by the veins when cervical connection is interrupted. It should be borne in mind that the formation of collateral lymph paths some time after the ligation of the thoracic duct is quite a different proposition from the question whether there exist lymphaticovenous anastomoses *functioning* under physiological conditions.

In our experiments, we attempted to find out whether dye infused intralymphatically (into a lymphatic of the leg) would appear in the blood in the presence of a thoracic duct fistula. Its appearance in the given conditions would have argued for the existence of lymph-

discussed this question and established the fact that fluid and smaller dye molecules may be filtered and diffuse through the walls of efferent lymphatics, to be reabsorbed through the blood capillaries. Besides, fluid may be reabsorbed in the lymph nodes as well. Let us remember Romhányi's observation who encountered absorptive vacuoles in the sinuses of fluid-filled lymph nodes. Our experiments, to be described in the following, furnish a further argument for the possibility that fluids and crystalloid molecules may escape from the lymphatics and — by-passing the thoracic duct — find their way into the blood path.



*Fig 181.* Perigastric lymph node in a case of gastric ulcer. Excessive lymph flow through the marginal sinus into the depth of the lymph node Absorptive vacuoles in the depth (photo by Romhányi)

Mention has already been made of our experiments in which dye and para aminohippuric acid was injected into a peripheral lymphatic of animals with thoracic-duct fistula. It is safe to assume that PAH, a substance of comparatively small molecules, is absorbed by the blood capillaries from the lymphatics in the same way as is water, provided such absorption is possible.




*Fig. 180.* Lymph nodes injected with India ink
1 = afferent lymphatics, 2 = efferent lymphatic; 3 = lymph node

## STORAGE OF FLUID IN THE LYMPHATIC SYSTEM

As has been noted in the preceding chapter, 13 per cent of the infused PAH was, on an average, recovered from the urine so that this amount must have been absorbed in the lymph nodes or passed from the lymphatics into the blood. In the same group of experiments, 7 per cent of the infused PAH was discharged by the thoracic duct. This means that 80 per cent of the substance — presumably together with the accompanying fluid, since lymph flow did not increase after the infusion of the fluid — must have remained somewhere in the lymph vascular system.

Storage of fluid in the valveless lymphatics of vertebrates of the lower orders is commonly known (Jossifov 1903, 1906; Bartels 1909): fluid accumulates in the lymph vessels and is then emptied towards the veins when the animal makes a sudden movement. Rouvière and Valette (1933) suggested the possibility that lymph nodes had the function of storing fluids and regulating lymph flow, but failed to prove this theory. They pointed to the fact that the amount of lymph pouring into the chyle vessels during digestion is in itself far in excess of what the thoracic duct is able to convey. They think that one of the tasks of the mesenteric lymph nodes is to temporarily retain and store part of the lymph. In its essentials, this theory is accepted by Zhdanov (1952). Horstman (1952), on the other hand, attributes a decisive role to the first postmural segment of the mesenteric lymphatics in the storage of fluid that has passed from the intestines into the lymph vessels. Fluid absorbed from the intestines pours from the parietal lymphatics suddenly into the mesenteric lymph vessels when the intestines contract. The mesenteric vessels are, however, unable to forward the whole amount of lymph so received, therefore part of it remains in their valvular segment where Horstman actually demonstrated pouchlike dilatations, the walls of which contained a reticulum composed of elastic fibres.

We share the view that lymph nodes play a certain role in fluid storage, a theory which is supported by the described experimental results as also by histological evidences.

Tremendously dilated sinuses containing serous fluid were histologically demonstrated (Romhányi) in certain parts of lymph nodes whose efferent lymphatic had been infused with fluid. Such lymph nodes had a spongy structure, and the distended sinuses occupied the major part of the lymph-node parenchyma.

But not only lymph nodes are capable of storing fluids. It has already been pointed out in this work that lymphatic vessels are exceedingly expansible: their cross section may grow to many times its usual size so that — in certain circumstances — great amounts of fluid may accumulate within the tubular system as well. Moreover, ampullar dilatations are not confined to the mesenteric lymphatics

bladder of the animals and collected the outflowing urine. The concentration o PAH as found in the urine is a reliable index of the quantity of the substance th has passed into the blood.

Table 50 summarizes the results of these experiments. On a average, 13 per cent of the infused PAH was discharged with th urine, so that at least this much had passed into the blood circulatio We must therefore assume that at least 13 per cent of the introduce substance was absorbed in the lymph nodes or diffused through th lymphvessel walls.

This, of course, still leaves the question open whether fluid i absorbed by blood capillaries in the lymph nodes or if dissolved mol cules and water diffuse through the lymph-vessel walls into th interstitial space to be absorbed there by the blood capillaries.

TABLE 50

| No | Infused PAH mg | PAH excreted by the kidneys mg | Percentage of infused PAH excreted by the kidneys |
|---|---|---|---|
| 1 | 10 5 | 1·0 | 9 5 |
| 2 | 66 0 | 12 8 | 19 5 |
| 3 | 79 0 | 3,8 | 4 8 |
| 4 | 81·5 | 16 8 | 20 6 |
| 5 | 124 0 | 13 0 | 9 2 |
| 6 | 57 2 | 6 0 | 10 5 |

We have briefly mentioned those indirect indications which poin to fluid being absorbed in the lymph nodes. Proofs in favour of filtration and diffusion from the lymphatics have also been discussed All these data seem to justify our conclusion that fluid absorptio into the blood capillaries takes place both in the lymph nodes an the lymph vessels. This means not merely an absorption of flui that has escaped into the interstitial space but — in agreement wit Zhdanov (1952) — *also a direct absorption of fluid from the lymphatic* by the blood capillaries. This, possibility, can, certainly not be disre garded: it was pointed out by Zhdanov that the efferent lymphatic are surrounded by a rich network of blood capillaries and that, thus it is quite conceivable that these capillaries absorb water from th lymph whose protein content is lower than that of the blood (provided conditions of capillary pressure are favourable).

# CHAPTER XI

## COMPOSITION OF LYMPH

It has repeatedly been emphasized that the fluid flowing in the lymphatics, i. e. the lymph, is not identical either with the capillary filtrate or with the "tissue fluid" and should be sharply distinguished from both. Fluids in the organism which belong to the "extracellular space" have, nevertheless, a very similar composition. Blood plasma, interstitial fluid, lymph and the fluids of the serous and synovial cavities can scarcely be distinguished by chemical analysis.

These fluids are separated only by thin endothelial and mesothelial membranes, permeable to water and dissolved molecules. Although the various membranes (wall of blood capillaries and lymphatics, serous and synovial membranes, etc.) may show different degrees of permeability, the size of their pores is such as to allow the passage of molecules dissolved in blood plasma so that the fluids on both sides of the membranes are in osmotic equilibrium. (In discussing filtration and diffusion through the wall of blood capillaries we pointed out that diffusion through the wall of blood capillaries is not "free" in the strict sense of the word, that — on the contrary — the size of the pores is such as to limit considerably even the diffusion of relatively small molecules, e g. of glucose which, however, does not alter the fact that the fluids on the two sides of the membrane remain in equilibrium. If this equilibrium is upset in some way, e. g through intravenous or intraperitoneal injection of water or hypertonic solutions, equilibrium will be re-established after a longer or shorter period.)

A detailed discussion of the composition of the different body fluids and their relationship to blood plasma would exceed the scope of the present work; numerous reviews of this subject are available. The work of Cameron (1945) for instance, contains a table in which not only the composition of the lymph but also that of the cerebrospinal fluid, the aqueous humour, the synovial and the amniotic fluid are compared with the composition of the blood plasma. Similar tables are to be found in almost all manuals and text-books (e. g. Ellinger (1902); Schulz (1925); Gerhartz (1925); Hammarsten (1926); Peters (1935); Evans (1945), etc.

It is shown by these data that the composition of all investigated fluids is very similar not only inter se but also in comparison with that of blood plasma, and that any differences are mainly attributable to the amount of protein contained in the different fluids. The permeability of the membranes separating the fluids is of such a nature as to hinder the diffusion or filtration of protein (colloid) molecules so that practically no complete equalization of the respective protein

(Horstman 1952): they occur also in numerous other lymph ve (Kubik 1952). Ampullae of this kind can, if they become disten take up quite a considerable amount of lymph. Lacking a satisfac method of measurement, it is hardly possible to determine the vol of fluid stored in this way in the lymphatics. Let us remember not even the total amount of fluid, i.e. lymph, contained in the lyn atic system under normal conditions has so far been measu Storey et al. (1951) attempted to make such measurements by m of radio-active proteins: the attempt remained unsuccessful, for - has been noted earlier in this work — only part of the extravas plasma proteins is contained in the lymphatics while their gre part is in the interstitial fluid. We have, therefore, to content ourse with estimated values, and we do not think that the total am of lymph is more than 1 to 2 l. This is, of course, not to mean only this much is transported by the lymphatic system, since amount drained from the thoracic duct per day is considerabl excess of this volume. The total amount of circulating plasma prot is filtered from the blood capillaries about once a day and is — together with an adequate volume of fluid — returned to the b path by the lymph vessels. The extent of this transport may bec much larger under pathological conditions. Let us refer in this nection to the experiments of Bollman et al.: the hepatic lymph ve were observed to have daily carried off 6 to 8 times the total am of circulating plasma proteins in cases of experimental cirrh

TABLE 52

*Physico-chemical properties of lymph (according to Bottazzi 1908)*

| | Serum | Thoracic-duct lymph | Cervical-trunk lymph | Brachial lymph | Chyle |
|---|---|---|---|---|---|
| Freezing-point | 0 595 | 0·615 | 0 612 | 0 623 | 0 610 |
| $K_{36°}$ C | $151\times10^{-4}$ | $162\times10^{-4}$ | — | $165\times10^{-4}$ | $157\times10^{-4}$ |

Beside the electrolytes (the concentrations of which show slight differences in accordance with Donnan's equilibrium), the organic molecules present in blood plasma are also present in the lymph in t[illegible]. It is not intended to quote here all relative [illegible] Heim's results [illegible] trunk [illegible] ed by [illegible] Meyer- and Mendel (1927), etc. The latest [illegible] (1953) who compared the compo- [illegible] duct with that of the blood plasma [illegible] ted (Table 54). [illegible] physico-chemical properties [illegible] plasma and lymph, [illegible] wing the concentrations [illegible] nitrogen, uric acid, creati- [illegible] gations concerning the concen[illegible] I

TABLE 53

*Relationship between the composition of the cervical-duct lymph and that of the blood plasma in the dog (according to Heim 1933)*

| | Plasma | | Lymph | | Number of animals |
|---|---|---|---|---|---|
| | limit values | average | limit values | average | |
| Protein | 5 54—7 23 | 6·18 | 1 38—4 57 | 3 32 | 16 |
| Residual-N | 21·1—46 0 | 32 6 | 19 8—45 4 | 34 8 | 10 |
| Carbamide | 17 9—28·0 | 21 7 | 19 8—33 0 | 23 5 | 7 |
| Uric acid | in traces | | in traces | | 3 |
| Creatine | 1 22—1 54 | 1 37 | 1 28—1 49 | 1 40 | 7 |
| Sugar | 112 0—143 0 | 123 0 | 107 0—144 0 | 132 0 | 16 |
| Amino-acids | | 4 90 | | 4 84 | 1 |

contents of the fluids on the two sides of the membrane can be established. Therefore, each particular fluid contains a different amount of plasma proteins according to the special properties of the separating membrane. These membranes are generally called "semi-permeable membranes"; however, we do not use this term as it is not true that crystalloids and dissolved molecules can freely pass through the membranes (by way of filtration or diffusion), whereas proteins, i.e. colloidal molecules are completely retained by them. By virtue of their special structure, biological membranes are not "completely permeable" for small crystalloid molecules and allow, on the other hand, passage of the larger protein molecules. The crystalloid composition of fluids is, however, influenced also by the permeability of the membrane to proteins. We are alluding here to the fact that the composition of lymph as well as that of other body fluids is determined by Donnan's equilibrium with the consequence that the sodium and potassium concentration of the lymph is lower and its chloride, bicarbonate and phosphate concentration higher than in the plasma (Table 51). It is due to the higher concentration of electrolytes that lymph shows a greater reduction of freezing-point and a higher conductivity than blood (Japelli and D'Erico (1907/08); Bottazzi (1908).

TABLE 51

*Comparison of electrolyte composition in the serum and the lymph of the cervical trunk (according to Drinker and Yoffey 1941)*

| | Serum | Lymph |
|---|---|---|
| pH | 7 34 | 7 41 |
| Protein g% | 6 2 | 3 3 |
| $H_2O$ g% | 93 8 | 96·7 |
| | maeq/l. | maeq/l. |
| Na | 163 | 157 |
| K | 4 | 3 5 |
| Ca | 6 | 4 |
| Mg | 2 | 1·5 |
| All cations | 175 | 166 |
| Cl | 125 7 | 126·3 |
| $HCO_3$ | 25 6 | 26 0 |
| Phosphate | 3 4 | 3 5 |
| Protein anions | 15·5 | 8·2 |
| Unknown anions | 4 8 | 2 0 |
| All anions | 175 | 166 |

TABLE 55

*Amino-acid concentration in different regions of the venous and lymphatic system*

| | Jugular vein | | Portal vein | | Thoracic duct | | Cervical trunk | | Liver lymph | |
|---|---|---|---|---|---|---|---|---|---|---|
| | average | limit values | average | limit values | average | limit values | average | limit values | average | limit values |
| Alanine | 32·8 | 19—52 | 35·6 | 27—53 | 30·7 | 17—47 | 35·2 | 28—44 | 29·6 | 22—43 |
| Arginine | 12·3 | 7—16 | 14·0 | 9—18 | 11·6 | 8—19 | 10·8 | 7—14 | 8·8 | 7—10 |
| Asparaginic-acid | 3·8 | 2—6 | 3·2 | 3—4 | 4·2 | 2—6 | 3·3 | 3—4 | 2·7 | 2—3 |
| Cystine | 12·2 | 6—21 | 8·8 | 5—16 | 10·2 | 5—16 | 12·3 | 5—19 | 6·0 | 4—7 |
| Glutamine | 58·2 | 38—88 | 50·7 | 34—72 | 55·3 | 32—80 | 63·0 | 42—85 | 46·2 | 33—70 |
| Glutamic-acid | 14·0 | 14 | 11·0 | 11 | 120 | 12 | — | — | 16·0 | 16 |
| Glycine | 11·8 | 8—20 | 13·8 | 9—17 | 10·3 | 6—14 | 12·5 | 6—21 | 11·6 | 8—18 |
| Histidine | 10·0 | 7—12 | 8·8 | 5—17 | 11·0 | 4—15 | 10·0 | 9—11 | 9·5 | 8—11 |
| Leucine+Isoleucine | 19·3 | 13—24 | 19·0 | 13—26 | 19·2 | 14—30 | 13·2 | 6—18 | 19·8 | 12—27 |
| Lysine | 11·2 | 7—16 | 10·0 | 5—17 | 8·5 | 4—19 | 9·0 | 7—12 | 6·6 | 3—10 |
| Phenylalanine | 9·5 | 6—12 | 10·2 | 7—14 | 12·7 | 6—21 | 7·8 | 7—10 | 11·6 | 8—19 |
| Proline | 19·0 | 17—22 | 19·5 | 16—23 | 15·0 | 12—20 | 15·0 | 10—20 | 15·0 | 15 |
| Serine | 9·0 | 6—12 | 7·4 | 5—11 | 9·6 | 5—14 | 10·7 | 8—13 | 8·5 | 6—11 |
| Threonine | 20·7 | 9—28 | 19·8 | 16—28 | 20·0 | 12—31 | 18·8 | 12—25 | 18·4 | 11—29 |
| Tryptophane | 8·5 | 6—13 | 9·3 | 8—11 | 14·7 | 11—17 | 6·7 | 6—8 | 14·7 | 11—18 |
| Tyrosine | 12·4 | 8—15 | 12·2 | 8—17 | 12·3 | 7—18 | 10·0 | 8—15 | 12·6 | 7—18 |
| Valine | 14·0 | 10—18 | 15·2 | 11—21 | 11·8 | 9—22 | 12·0 | 7—17 | 15·4 | 12—19 |

TABLE 54

*Comparison of human thoracic-duct lymph and blood plasma (according to the data of Bierman and associates 1953)*

| | Plasma | | Lymph | | Number of examined persons |
|---|---|---|---|---|---|
| | limit values | average | limit values | average | |
| Residual N | 15·8—14·10 | 48 8 | 13·4—139 0 | 46 5 | 5 |
| Uric acid | 1·6—10·9 | 5·1 | 1·7—10 8 | 5 0 | 4 |
| Creatinine | 0 8—9.0 | 3·0 | 0 8—8 9 | 3 0 | 5 |
| Total cholesterol | 83—167 | 117 0 | 34—106 | 68 | 5 |
| Free cholesterol | 28—56 | 38 | 15—51 | 34 | 5 |
| Glucose | | 117 | | 136 | 1 |
| Bilirubin | 0 6—1·1 | 0·8 | 0 9—1·1 | 1 0 | 2 |
| Alk phosphatase | 1·7—7·3 | | 6·3—21·0 | | 2 |
| Total protein | 5 4—9 4 | 7 1 | 2·9—7 3 | 4 9 | 5 |
| Albumin | 2 0—3 5 | 2 9 | 1 5—2·7 | 2 3 | 5 |
| Globulin | 3 1—6·9 | 4 2 | 1 5—4 8 | 2 8 | 5 |
| Sodium | 113—135 | 127 | 118—132 | 127 | 5 |
| Potassium | 4 1—5 9 | 5·0 | 3 9—5 6 | 4 5 | 5 |
| Calcium | 8 5—11 8 | 10 0 | 6 8—11·1 | 8 8 | 5 |
| Chlorine | 94—98 | 96 0 | 87 0—103 0 | 96 | 5 |
| Phosphate | 3·7—6 3 | 4 5 | 3 6—6 4 | 4·4 | 5 |

plasma and lymph. As is known, blood contains free amino-acids not attached to proteins. It can be assumed that it is chiefly these substances, of small molecular size and therefore diffusible, which are at the disposal of the cells for the synthesis of their proteins. If, therefore, the tissues — according to their metabolic requirements — take up a larger amount of some amino-acid, the concentration of that particular amino-acid can be expected to decrease in the lymph. If, on the other hand, protein is broken down the free amino-acid content of the lymph can be expected to increase. Not having found any report on this subject, it seemed to us worthwhile to investigate the role which the lymph plays in the transportation of amino-acids.

... for 16 hours were ... t and col- ... itic lymph ... lrew blood ... 's the concentration of amino-acids by the method of quantitative paper chromatography described elsewhere (Kisfaludy and Braun) 1954; Braun, Kisfaludy and Dubszky 1955)

observed in individual cases do not allow inferences as to the metabolism of the amino acids.

It should be mentioned here for the sake of completeness that, according to Burton-Opitz and Nemser (1916), the relative viscosity of the lymph of the thoracic duct amounts to about 1·7, and its specific gravity to 1012—1023 (1016, on average). Of course, both values vary according to the composition of the lymph. Drinker and Yoffey (1941) published a table showing the data of different authors regarding the freezing-point of lymph. Their mean value is — 0·60°C (—0·526 to 0·690°C).

As we have seen, the composition of the lymph does not substantially differ from that of the blood plasma whether we consider the electrolyte ions or the dissolved inorganic and organic molecules contained in it. The only considerable difference is that regarding their respective protein concentrations: lymph generally contains much less protein than blood plasma. But there is a great difference also in the protein level of lymph collected from different areas. It makes a great difference whether we analyse the mixed lymph of the thoracic duct or that drawn from the efferent lymphatic of some other organ or region.

Drinker and Yoffey (1941) established the order of the protein level of lymph derived from various regions: 1. lymph from the liver and gall bladder; 2. lymph from the thoracic duct; 3. lymph from the heart, kidney and intestines; 4. lymph from the lung and cervical trunk; 5. lymph of the skin and the subcutaneous connective tissue.

The differences observed in the protein content of the lymph from various regions are due to differences in the permeability not of the lymphatics, but of the blood capillaries. The protein content of the lymph flowing from a particular lymphatic and the appearance of intravenously injected colloids in the lymph allow, therefore, certain inferences to be drawn as to the permeability of the blood capillaries in that area.

Results obtained from the observation of the appearance in the lymph of colloidal substances introduced into the circulation are in perfect agreement with the data obtained from the examination of the protein content of the lymph collected from different regions. Intravenously administered colloids appear most quickly in the hepatic and most slowly in the peripheral lymph (i.e. that collected from the extremities.) Investig[illegible]
with labelled plasma [illegible]
used as a substitute f[illegible]

The results of these experiments (performed on 6 dogs) are summarized in Table 55. Highest was the concentration of glutamine, and than following in descending order: alanine, threonine, leucine + isoleucine.

The concentration of amino-acids in the blood serum and in the lymph drawn from various regions showed no striking divergences; the same amino-acids ere encountered in lymph and blood alike. While, as just mentioned, the average concentration of the various amino-acids in blood and lymph did not essentially differ, striking differences — exceeding the margin of error inherent in the method — were observed between particular experiments. The concentrations of the various amino-acids of the lymph drawn from *different regions*, as also those of the blood plasma revealed differences of 50 and even 100% (Table 56). Since these differences showed no uniform tendency in the different experiments, no far-reaching conclusions can be drawn from them.

TABLE 56

*Amino-acid concentration g/ml*

| Fluid examined | Alanine | Arginine | Asparagine | Cystine | Glutamine | Glycine | Histidine | Leucine | Lysine | Phenylalanine | Proline | Serine | Threonine | Tyrosine | Tryptophane | Valine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Serum* | | | | | | | | | | | | | | | | |
| Jugular vein | 52 | 16 | 6 | 9 | 61 | 20 | 12 | 18 | 16 | 12 | 19 | 12 | 28 | 15 | — | 18 |
| Portal vein | 53 | 18 | 3 | 6 | 62 | 13 | 17 | 13 | 17 | 14 | 23 | 11 | 28 | 12 | 11 | 16 |
| *Lymph* | | | | | | | | | | | | | | | | |
| Thoracic duct | 47 | 12 | — | 5 | 46 | 13 | 10 | 14 | 10 | 14 | 12 | 5 | 26 | 15 | — | 16 |
| Cervical trunk | 41 | 9 | 3 | 5 | 42 | 21 | 11 | 13 | 7 | 7 | — | 8 | 25 | 8 | 6 | 11 |
| Liver lymph | 43 | 10 | 3 | — | 42 | 18 | 8 | 15 | 5 | 9 | — | 11 | 21 | 9 | — | 12 |

As has been pointed out lymph is in osmotic equilibrium with the interstitial fluid and the blood plasma so that the concentration of the substances of small molecular size is essentially equal in lymph and plasma. Hence, when the cells take up amino-acids from or release amino acids to the interstitial spaces, these acids (in the same way as, for instance, glucose) will diffuse from points of higher to those of lower concentration, so that differences of concentration are quickly balanced. It is thus understandable that in our experiments the average amino-acid concentration of the lymph draining from different regions (which represented the interstitial fluid of these regions in this respect) did not substantially differ from that of the plasma. The differences

TABLE 57

*The ratios of lymph and plasma concentrations of colloidal indicator substances 2 hours after intravenous injection, compared with the relative protein content of the lymph*

| Origin of lymph | Protein concentration of lymph % | Authors | Injected substance | Concentration in the lymph % | Authors |
|---|---|---|---|---|---|
| Thoracic duct | 67 | Saito and Nakazawa 1932 | Human-albumin | 53 | Krieger et al. 1950 |
| | 64 | Field et al. 1934/35 | Dog plasma | 40 | « « |
| | 55 | Nix et al. 1951 | Human-albumin | 67 | Wassermann and Mayerson 1952 c |
| | 58 | Rényi-Vámos 1954 | autologous serum labelled with Evans-blue | 81 | Szabó 1954 |
| | | | Dextran 50 000 | 77 | « « |
| | | | Dextran 87 500 | 52 | Wassermann and Mayerson 1952 b |
| | | | Dextran 125 000 | 63 | « « |
| | | | Dextran (20 ml/kg) | 65 | Bollman 1953 |
| Cervical trunk | 57 | Field et al. 1934/35 | autologous serum labelled with Evans-blue | 21 | Szabó 1955 |
| | | | Dextran 50 000 | 11 | « 1954 |
| Liver | 7 | Field et al. 1934/35 | autologous serum labelled with Evans-blue | 71 | Szabó 1954 |
| | 84 | Nix et al. 1951 | Dextran 50 000 | 86 | « « |
| | 82 | Rényi-Vámos 1954 | Dextran (20 ml/kg) | 75 | Bollman 1953 |
| | 75 | Friedman et al 1955 | | | |
| Intestine | 50 | Wells 1932 | autologous serum labelled with Evans blue | 59 | Szabó 1954 |
| | 49 | Nix and co-work. 1951 | Dextran 50 000 | 45 | « « |
| | 41 | Rényi-Vámos 1954 | Dextran (20 ml/kg) | 15 | Bollman 1953 |

*case (increase of the average diameter of pores or of the total pose surface, decrease in the pressure of filtration and a consequent diminution of filtration flow of fluid, etc.) cannot, of course, be ascertained from the protein concentration of the lymph alone.*

The penetration of colloids from the blood plasma into the lymph can, in principle, be measured with different methods. According to Wassermann and Mayerson (1952), for example, the establishment of equilibrium between blood plasma and lymph which takes, on average, some 8 hours is reduced to about 80 minutes after the administration of 100 ml of 25% radio-active albumin solution. However, the rate of equilibration also depends on the amount of the injected substance. The data indicated in Table 57 refer to experiments in which the substance was administered in tracer doses or at least in negligible quantities.

The method employed by Wassermann and Mayerson cannot be applied in every case. It is at any rate simpler to ascertain the ratio which exists at a given time between the concentration of the blood plasma and that of the lymph. The duration of such analysis must not be too short as, otherwise, results will show a great dispersion, nor must it last too long as otherwise the concentrations both in blood and lymph will have become too low, and also various other errors may result. According to our experiences, it is advisable to compare concentrations in blood and lymph 2 hours after the intravenous injection. Table 57 contains several data of this kind (they are partly our own and partly those of other authors).

It should be noted that the results presented in the table are not completely in accord with those of Grotte, Knutson and Bollman (1951) and Grotte (1955). These authors investigated the dextran content of the lymph of the thoracic duct after the intravenous injection of dextran solutions with different molecular weights. The dextran *concentration of the lymph reached its highest level after* 3 hours, or earlier, even after the i.v. injection of dextran with the highest molecular weight (205 000). With the exception of the dextran fraction with a molecular weight of 205 000 in which case the dextran concentration of the lymph amounted to 82% of that of the blood, 3 hours after the injection the concentration in the lymph of all other dextran fractions was as high or even higher than in the blood. For instance, in the case of a fraction with a molecular weight of 52 000, the dextran concentration in the lymph rose after 3 hours to 137 per cent of that of the blood.

These results may have been influenced by the fact that the amounts of introduced dextran were comparatively large (rats with an average weight of 200 g received 5 ml of the 6% solution). Besides, lymph which had been collected during a longer time was not compared with the average value of the concentration of the blood drawn during the same period but with that of blood drawn after the collection of lymph. By that time, concentration in the blood had presumably more or less decreased. Finally, it seems that the authors determined the

As the electrophoretic spectrum of the lymph contains the same protein fractions as the serum, it is not astonishing that lymph — from whatever region it originates — coagulates in a comparatively short time. Howell (1914) demonstrated the presence of fibrinogen and prothrombin but found little thrombokinase in the lymph of the thoracic duct. Also Drinker and Yoffey (1941) confirm that lymph contains but little thrombokinase, the reason of this phenomenon being that the lymphocytes, i.e. the most important cellular elements of the lymph, show little thrombokinase activity. We, too, established (unpublished experiments carried out together with Horányi) that the lymph of the extremities, the liver and even that of the thoracic duct contained practically no platelets. According to Brinkhous and Walker (1941), the fibrinogen content of the lymph of the thoracic duct amounts to 0·21% (43 to 71% of that of the plasma, with an average of 51%). The average prothrombin concentration is 51% (32—65%) of that of the plasma. The average prothrombin level of the portal lymph, however, is 93% (67—125%), that of the lymph of the extremities only 7·6% (5·8—9·3%) of the values ascertained in the plasma. According to Mann, Mann and Bollman (1949), if a complete intestinal lymph fistula is produced in rats, i. e. if the passage of all intestinal lymph into the blood plasma is prevented, marked hypoprothrombinaemia will appear very soon (after 24 hours, as a rule), which may be inhibited by the parenteral administration of vitamin K. *As long as the lymphatic fistula exists it is not possible to stop* hypoprothrombinaemia for long by means of blood transfusion. The

TABLE 58

*Protein content of human lymph*

| Origin of lymph | Number of experiments | Total protein | | Albumin | | Globulin | | A/G | | Authors |
|---|---|---|---|---|---|---|---|---|---|---|
| | | S | L | S | L | S | L | S | L | |
| Thoracic duct | 5 | 7·1 | 4·9 | 2·9 | 2·3 | 4·2 | 2·8 | 0·70 | 0·82 | Bierman et al. 1953 |
| | | | 2·8 | | 1·6 | | | | | Courtice et al. |
| | | 6·0 | 3·6 | 3·5 | 2·4 | 2·5 | 1·2 | | | 1951 |
| | | | | | | | 2·0 | | | |
| Testis | 4 | — | 2·9 | — | — | — | — | 1·1 | 1·31 | Rényi-Vámos 1954 |
| Penis | 1 | 5·1 | 3·8 | — | — | — | — | 1·56 | 1·42 | Rényi-Vámos 1954 |
| Elephantiasis of skin | 1 | 7·7 | 5·3 | — | — | — | — | — | — | Földi 1954 |

dextran in the whole blood and not in the blood plasma, a wrong procedure, since colloidal dextran does not penetrate into the red blood corpuscles so that dextran concentration is much lower in the whole blood than in the plasma alone.

Grotte (1955) studied the penetration of dextran fractions with a molecular weight of 4000 to 350 000 — into the peripheral lymph (*of the extremities*) *of dogs. Dextran with a molecular weight of 600* penetrates freely into the lymph (L/B concentration quotient = 1·0), in case of higher molecular weights (10 000 to 40 000), the L/B quotient remains low even after equilibration, i.e. the dextran level of the lymph does not reach that of the blood plasma. The quotient gradually diminishes with increasing molecular weight. In dextran fractions of high molecular weight (60 000 to 350 000), the L/B quotient is very low, but independent of the molecular weight. Grotte draws from this the far-reaching conclusion that the larger colloidal molecules, and so also plasma proteins, are not filtered — nor do they diffuse — *through* the pores of the capillary wall: these pores must be so small as to retain dextran molecules with a molecular weight of above 40 000 as also serum albumin; these colloids are leaking through openings produced by injury of the capillary wall.

Grotte finds this theory to be substantiated also by his own paper-electrophoretic experiments in which A/G quotients in lymph and blood plasma were identical within the margins of error. These results are in contradiction to numerous other observations so that we cannot quite accept the conclusions drawn from them. Contrary to the results of Grotte, the lymph generally contains somewhat more albumin and less globulin. Consequently, the A/G index is higher than in the blood plasma. Drinker and Yoffey (1941) published tables showing the protein concentration of the lymph of different animals, as well as data about the lymph collected from various regions within the same species. Making use of the tables of Drinker and Yoffey, we also publish two lists of this kind based on available information of a more recent date regarding man and dog. (Tables 58 and 59).

*Rényi-Vámos (1954) fractionated the lymph derived* from various regions with paper electrophoresis and compared their protein spectrum with that of blood plasma. The results showed that the lymph of both man and dog contained all serum protein fractions Electrophoresis showed further that the A/G quotient was usually higher in the lymph than in the serum.

Friedman, Byers and Omoto (1956) examined the liver lymph of rats. Average daily lymph production amounted to 3·0 ml and the average protein content of the lymph was 30 per cent less than that of the plasma. In their electrophoretic experiments, the ratio albumin to globulin was higher in the lymph than in the plasma (lymph: 1·0, plasma 0·9) which means that the lymph contains relatively somewhat less globulin. Apart from this quantitative difference, the electrophoretic curves of plasma and lymph were very similar.

TABLE 59

*Protein content and colloid-osmotic pressure in the normal dog*

| Origin of lymph | Number of animals | Prot g% | | Alb g% | | Globulin g% | | A/G | | Colloid-osmotic pressure cm $H_2O$ | | Authors |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | S | L | S | L | S | L | S | L | S | L | |
| Thoracic duct | 20 | 7·31 | 4·88 | — | — | — | — | — | — | 26·6 | 17·2 | Saito and Nakazawa 1932 |
| | 19 | 7·55 | 4·29 | — | — | — | — | — | — | — | — | Meyer-Bisch and Günther 1925 |
| | 13 | 6·25 | 4·00 | 3·61 | 2·45 | 2·63 | 1·54 | 1·46 | 1·72 | 30·6 | 19·1 | Field et al. 1934/35 |
| | 6 | 5·97 | 3·23 | 3·33 | 2·04 | 2·18 | 0·88 | 1·62 | 2·41 | — | — | Nix et al. 1951 |
| | 9 | 5·78 | 3·92 | — | — | — | — | 0·89* | 0·91* | — | — | Rényi-Vámos 1954 |
| Cervical trunk | 13 | 5·25 | 3·63 | 3·61 | 2·36 | 2·63 | 1·26 | 1·46 | 1·96 | 30·6 | 16·0 | Field et al. 1934/35 |
| Liver | 4 | 6·34 | 5·32 | 3·38 | 2·89 | 2·96 | 2·51 | 1·21 | 1·23 | 30·1 | 17·4 | Field et al. 1934/35 |
| | 13 | 5·67 | 4·39 | 3·41 | 2·74 | 1·81 | 1·28 | 2·04 | 2·32 | — | — | Nix et al. 1951 |
| | 13 | 6·78 | 5·32 | — | — | — | — | 0·87* | 0·91* | — | — | Rényi-Vámos 1954 |
| Intestine | 12 | 5·98 | 2·97 | 3·18 | 1·72 | 2·80 | 1·25 | 1·17 | 1·39 | 25·6 | 12·5 | Wells 1932 |
| | 10 | 5·67 | 2·79 | 3·47 | 1·90 | 1·62 | 0·64 | 2·31 | 3·18 | — | — | Nix et al. 1951 |
| | 10 | 6·43 | 3·63 | — | — | — | — | 0·82* | 109* | — | — | Rényi-Vámos 1954 |
| Kidney | 1 | 8·02 | 3·79 | — | — | — | — | — | — | — | — | Drinker and Field 1931 |
| (Kidney capsule) | 3 | 6·79 | 3·45 | — | — | — | — | — | — | — | — | Foldi and Szabó 1952 (unpubl.) |
| | 3 | 5·50 | 3·80 | — | — | — | — | 0·94* | 1·19* | — | — | Rényi-Vámos 1954 |
| Testis | 5 | 6·80 | 3·83 | — | — | — | — | 0·90* | 1·21* | — | — | Rényi-Vámos 1954 |
| Heart | 6 | 5·95 | 3·83 | 2·98 | 2·20 | 2·96 | 1·64 | 1·12 | 1·39 | 23·4 | 17·5 | Drinker et al. 1940 |
| *Lung* | *18* | — | 3·63 | — | — | — | — | — | — | — | — | *Warren and Drinker* 1942 |
| Leg | 8 | 6·46 | 1·91 | 3·72 | 1·20 | 2·84 | 0·71 | 1·38 | 1·81 | 30·3 | 10·0 | Field et al. 134/35 |
| | 6 | 6·55 | 2·42 | — | — | — | — | — | — | — | — | Szabó 1954 |
| | 6 | 6·15 | 1·08 | — | — | — | — | 0·91* | 1·19* | — | — | Rényi-Vámos 1954 |

authors conclude from their experiments that vitamin K, required for the production of prothrombin, is absorbed from the gastro-intestinal tract exclusively through the lymphatics.

Very interesting questions arose in connection with investigations concerning the lymphatic concentration of antibodies and immune bodies. It is well-known that the immune bodies which circulate in the plasma, together with other proteins, gain access to the lymph. (Hughes and Carlson [1908]; Becht and Luckhardt [1916]). This is a matter of course, for, if all protein fractions pass from the plasma into the lymph, why should immune globulins behave differently? We know, however, from Takahashi's experiments (1933), and especially from those of McMaster and Hudack (1935) that lymph nodes play an important role in the production of antibodies. These authors demonstrated that, very soon after the intracutaneous injection of various bacteria, a high specific agglutinin titer appeared in the regional lymph nodes of the ear of mice. It is probable that the antibodies formed in the lymph nodes are mostly brought into the blood stream by the lymph vessels.

In all probability, matters are quite similar in respect of certain endocrine glands. Their protein-like secretions are presumably first absorbed by the lymphatics, and it is then via the thoracic duct that they reach the blood. However, as no reliable evidence is available to substantiate this assumption, it would be premature to speak of a "lymphocrine" system. At any rate, earlier researches point to the probability that insulin from the pancreas reaches the circulation through the lymphatics. The lymph of the thoracic duct is supposed to lower the level of blood sugar. The value of these earlier researches seems to us rather doubtful, partly because the methods employed were not reliable, and partly because the molecular size of the insulin itself is not large enough to prevent its absorption into the blood capillaries. Besides, in the course of our own experiments we encountered no lymph capillaries in the islets of Langerhans (Földi, Kepes and Szabó (1955).

Ottaviani (1947) raises the question whether it is possible to speak of "lymphocriny" in connection with the function of the hypophysis. In his opinion, the hormones of the hypophysis, at least those of the anterior lobe, are secreted into the lymph. He sees a proof of this hypothesis in the observation that, after the ligature of the cervical lymphatics, dilated lymph vessels and certain histological changes are observable in the anterior pituitary lobe, and that in the lumen of the lymph vessels "rods" of the same kind are observed as are encountered

[illegible] thyroid as
[illegible] ase dilated
[illegible] hich stains
[illegible] stantiating

of the thoracic duct was found to be 4·18 (±0·11) g% and that of the cervical trunk 3·07 (±0·18) g%. Bollman (1951) too, assumes that the total amount of circulating plasma proteins passes through the lymphatic system of the animal in 24 hours.

Shafiroff and his co-workers suggest that, after loss of blood, the lymphatic system plays an important part in restoring plasma proteins. They report (Shafiroff et al. 1943) that, after haemorrhage, the protein content of the lymph increases and thus contributes to the regeneration of plasma proteins. It was found in the course of further experiments (Co-Tui et al. 1949) that, after loss of blood, the restoration of normal plasma protein level was substantially retarded if the thoracic duct was ligated. Whereas, in normal animals, the protein level of the blood plasma returned to normal even after a great haemorrhage in 24 to 48 hours, this process was not complete even after 8 days in animals with occluded lymphatic trunks. We can see that — according to these authors — the lymphatic system plays an important role in conveying mobilized proteins into the circulation. An occlusion of the thoracic duct and especially a fistula constitutes, therefore, a serious obstacle for the entry of extravascular proteins into the circulation.

Lymphatic fistula is of a fairly frequent occurrence in man. Numerous publications describe lymphatic fistulae induced by traumatic or operational injury of the thoracic duct as also by tumorous erosion. It is, however, fairly difficult to produce experimental fistula through which lymph is flowing for any length of time. That the experiment is so difficult is principally due to a coagulation of the lymph in the cannula. Recently, Glenn et al. investigated (1949) in dogs, Bierman et al. (1953) in man, the effect of an experimental chronic fistula of the thoracic duct. In the six experiments of Glenn and his associates the average protein level of the plasma dropped in 3—5 days from 6·11% (5·43—7·08%) to 3·84% (2·71—4·22%). It fell, in a case of Bierman and his co-workers, from 6·31% to 4·52% in two days.

Ehrenhaft and Meyers (1948) were justified in pointing to the dangers of lymphatic fistula and shared the view of Drinker and Yoffey that an occlusion of the thoracic duct was better for the patients than a fistula of this organ. Any injury of the thoracic or abdominal section of the thoracic duct involves the danger of chylothorax, chylous ascites which, like the fistula of other larger lymphatics, leads to progressive inanition of the patients. According to Shackleford and Fisher (1938), the mortality rate of chylothorax caused by the injury, of the thoracic duct is 50 per cent. A surgical ligation of the thoracic duct on the other hand, does not, as a rule involve lasting consequences; Munk and Friedenthal (1901), Lee (1922) and others succeeded in demonstrating that the view, according to which a ligature of the thoracic is usually fatal, is completely unfounded. Ligation of the thoracic duct causes lymph congestion, and increased hydrodynamic pressure promotes the opening or dilatation of collaterals and even the formation of new

and Szabó 1955). Buno (1946) demonstrated the presence of colloid also in the efferent regional lymph nodes of the thyroid gland.

We shall deal with these researches elsewhere in more detail; what we would emphasize here is that the fact that the presence of a protein which stains like colloid has been demonstrated in the lymphatics of the thyroid is not necessarily evidence to show that the active principle of the thyroid secretion reaches the blood via the lymphatics. It is possible that the thyroxine is detached from the protein molecule and that it passes into the blood stream independently. We found in the course of our (unpublished) experiments that the lymph of the cervical trunk did not contain more organic iodine than the blood which was drawn at the same time from either the carotid artery or the jugular vein. However, not even this observation suffices to decide the problem definitely because it shows only the momentary situation, and it is quite possible that — at a given moment — no organic iodine (thyroxine) is released by the thyroid.

It is, on the other hand, known that organic iodine is adsorbed to plasma protein. One would, therefore, expect that the lymph of the cervical trunk which has a lower protein level than the blood plasma contains also less organic iodine than the blood plasma, provided no thyroxine is released by the thyroid gland. Since in our experiments the iodine level was practically equal in the blood plasma and the lymph of the cervical trunk, it seems probable that thyroxine passed from the thyroid gland into the lymph. It is questionable, however, *whether this is the principal route by which thyroxine enters* the circulation.

The lymph has, as we have seen, a fairly high protein content. In man, the lymph of the thoracic duct contains about 5% protein, on an average. At least 1 to 2 litres of lymph are drained from the thoracic duct every day. If this lymph does not get back into the blood circulation (injury or fistula of the thoracic duct) it means a very great loss of plasma proteins, amounting to 50—100 g per day. The patient becomes very rapidly hypoproteinaemic, though the degree of hypoproteinaemia cannot be taken as a measure of the actual loss of plasma proteins since the volume of circulating plasma will diminish and give rise to haemoconcentration.

Forker, Chaikoff and Reinhardt (1952) observed that in dogs, for instance, 14·4 g of plasma proteins flowed daily through the cannula tied into the thoracic duct, whereas the whole amount of circulating protein amounted to 34 g. These experiments were performed on anaesthetized animals in which lymph circulation is substantially less than in conscious animals. They found that rats had lost 10 7 g of protein in 11 days: this means a daily loss of almost 1 g, i. e. more than the total amount of the animal's circulating plasma proteins. In an earlier publication Reinhardt and Li (1945) reported that, on an average, 390 (±30) mg flowed from the thoracic duct and 38·5 (±3·8) mg from

TABLE 60

*Change in the composition of chyle after food ingestion (according to Lehnartz 1940)*

| Hours after feeding | Protein % | Carbohydrate % | Fat % |
|---|---|---|---|
| 0 | 3·11 | 0·095 | 0·22 |
| 2 | 3·49 | 0·126 | 0·24 |
| 4 | 3·07 | 0·161 | 2·52 |
| 6 | 3·13 | 0·164 | 3·86 |
| 8 | 2·76 | 0·205 | 2·18 |

with chyle after the uptake of food. We would refer here to the chapter on the special physiology of the lymphatics of the gastrointestinal tract in which the question of fat absorption is discussed in detail.

Up to a certain point, the carbohydrate (glucose) concentration of the mesenteric lymph may also increase after the ingestion of food, but it seems to be quite certain that the lymphatic system plays no decisive part in the intestinal absorption of sugar. Benson and his associates (1956) examined the role played by the lymph paths in the transport of water and salt absorbed from the intestines. They found that none or only an infinitesimal part of the $D_2O$ or $Na^{24}Cl$ absorbed from the intestines passed into the mesenteric lymphatics. At least 99 per cent of the introduced amount is removed by the portal circulation. Whether they had been administered perorally or intravenously, all the labelled water and salt demonstrable in the lymph gained access to the lymphatic paths via the arterial blood, from the blood plasma.

According to available data, the lipid content of the lymph of the thoracic duct originates from the mesenteric lymphatics (Dabelow (1930/31); Drinker and Yoffey (1941). Lymph arising from all other regions is clear like water, and does not contain fat "chylomicrons", in larger quantities, which are the cause of the turbidity of chyle.

It seems, however, that as lipids pass from the lymphatic system into the blood stream, so can they pass also from the blood circulation into the thoracic duct lymph. Reinhardt, Fisher and Chaikoff (1944), after the intravenous injection of lipids labelled with radioactive phosphorus, succeeded in recovering 9 to 20% of them from the thoracic duct within 3 to 6 hours. Glenn and his associates (1949) showed that the total lipid concentration of the lymph increased very markedly if animals with a fistula of the thoracic duct were given fluid through a gastric tube (e.g. in one of the cases from 260 to 821 mg% in 38 minutes). It was indifferent from this point of view whether the perorally administered fluid was water, glucose or saline solution. The authors think of the possibility (not regarded, however, as probable even by themselves) that the lipids stored in the lacteals are washed out by the

lymphatico-venous anastomoses, so that proteins and other substances that have entered the lymph channels are returned into the blood stream. None of these mechanisms is operative in cases of lymphatic fistula so that lymph is lost from the body which means a considerable loss of water, electrolytes, and proteins. It is for this reason that almost all authors (v. Drinker and Yoffey (1941); Ehrenhaft and Meyers (1948) recommend that, whenever the thoracic duct is damaged, it, and especially its distal end, should be tied off because it can be expected that the ligature will promote the formation of corresponding collaterals and the cessation of the patients' dangerous condition.

We have seen the significance of the lymphatic system in the maintenance of an adequate plasma protein level. Still more important is, however, the role of the lymph vascular system in fat metabolism, in the intestinal absorption of fats.

Asellius described as early as 1627 that lymph (chyle) turned white, opaque and milky after the ingestion of food. This phenomenon has always been explained by the absorption of fat. According to Hunter (1784), the lymphatic system appears to be the only pathway of intestinal fat absorption. Munk and Rosenstein succeeded, as is described in their classical work (1891), in recovering, through a lymphatic fistula which one of their patients had on the leg, 60 per cent of the fat ingested. Bloom and his co-workers (1950) recovered with the lymph of the thoracic duct 92 per cent of the fatty acids labelled with $^{14}C$ they had perorally administered to unanaesthetized rats. This would mean that not more than 8 per cent would have been carried off by the lymphatico-venous anastomoses or the portal vein. The absorption of fat, in itself, does not necessarily increase the volume of mesenteric lymph flow nor the amount of the lymph in the thoracic duct, [illegible] Neither does the [illegible] the mesenteric [illegible] is a product of capillary filtration; the only change it undergoes consists in a considerable increase of its lipid content (Table 60).

Bollman et al. (1950) re-examined the composition of the lymph of the thoracic duct, the intestinal- and the hepatic lymph after fatty diet. Their results were in essential agreement with earlier ones. Whereas the aggregate lipid content of the lymph of the thoracic duct (averaging 626 mg% in starving animals) does not essentially change after the ingestion of fat-free meal, this value is multiplied after the consumption of fat and reaches its maximum in about 6 hours. Not only the concentration of neutral fats, but also that of phospholipids increases in the lymph, while the concentration of cholesterol changes but slightly. The fat content of the liver lymph does not increase after the ingestion of fat except if there exists a communication between the intestinal lymph vessel and the hepatic lymphatics above the point where the cannula for the collection of lymph is inserted. The intestinal lymphatics, on the other hand, are white, like milk, and engorged

TABLE 69

*Change in the composition of chyle after food ingestion (according to Lehnartz 1910)*

| Hours after feeding | Protein % | Carbohydrate % | Fat % |
|---|---|---|---|
| 0 | 3 11 | 0 095 | 0 22 |
| 2 | 3 49 | 0 126 | 0 24 |
| 4 | 3 07 | 0 161 | 2 52 |
| 6 | 3 33 | 0 164 | 3 86 |
| 8 | 2 76 | 0 205 | 2 18 |

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great amount of fluid. Rony, Mortimer and Ivy (1932) found high lipid concentration in the lymph of the thoracic duct even in dogs that had been starved for 2 to 14 days. This phenomenon was earlier described by Heidenhain in 1888. However, the concentration of lipids ceases to increase after the resection [illegible] and his associates, as well as [illegible] fat, mobilized during starvati[illegible] the intestinal mucosa which secretes it into the lumen of the intestines. The major part of the fat secreted gets into the intestinal lymphatics, and from there back into blood circulation through the thoracic duct.

Kim, Bollman and Grindlay (1956) claim that the turnover of this internal circulation of lipids is fairly constant. In rats, through the intestinal lymphatics, in dogs through the thoracic duct, a constant daily amount of lipids returns to the blood stream during starvation or fat-free diet. The observed total daily excretion of fatty acids amounted to 50 mg/100 g in rats with thoracic-duct fistula, and to 220 mg/kg in dogs. The volume of the excreted fatty acids was reduced to about 1/2 if the dogs underwent pancreatectomy, if the pancreas was cannulated or if the bile duct was tied off. The continuous evacuation is regarded by the authors as an indication of the fact that a constant [illegible] the intestines. [illegible]

It is, on the other hand, claimed by Pessoa et al. (1953) that — in the absence of bile and pancreatic fluid — the faecal discharge of endogenous lipids increases three- to fourfold. The connection is thus clear: under normal conditions, a great part of the lipids, 2/3 to 3/4 of the amount excreted in the intestines is reabsorbed and removed by the lymphatics, while the rest is discharged with the faeces. If, however, the absorption of the lipids is disturbed, that part is evacuated too, which is otherwise returned by the lymphatic apparatus.

We see that the lymph contains considerably more lipids after the peroral ingestion of food: the lipid level reaches its peak within 4 to 8 hours. We have also seen that the principal, if not only, way for the [illegible]

circulation. The ligation of the thoracic duct has — as was demonstrated by Lee in 1922 — only an uncertain and temporary effect due to the presence of collaterals, new lymphatico-venous anastomoses, etc. Ehrenhaft and Meyers (1948) tied off the thoracic duct of a patient and then compared the values obtained after starvation with post- [illegible] the operation [illegible] average values. [illegible] level of the blood remains unchanged in dogs with thoracic-duct fistula after the

peroral administration of fat (4 ml/kg). This proves that lymphatic fistulae are dangerous from a nutritional point of view because they practically stop fat absorption. With reference to Clarke, Goodman and Ivy (1918), we should point out that a resection of all available mesenteric lymph nodes, i.e. a single experimental obliteration of the intestival lymph paths, does not suffice to produce a lasting disturbance of fat absorption. Alimentary hyperlipaemia temporarily decreases after operations but this phenomenon disappears within six days. The authors explain this by the rapid regeneration of the lymph ducts. At the autopsy of the animals they found that in six of the operated ten dogs not only the lymphatics but also the extirpated lymph nodes had regenerated.

Chaikoff et al. (1951, 1952) demonstrated that different lipids — including cholesterol — are absorbed from the intestines mainly through the lymphatics. In rats, for example, they found in the thoracic duct 94 to 100 per cent of the labelled cholesterol absorbed from the intestines. Though in man, under conditions of starvation, the cholesterol level of the lymph is lower in normal circumstances than that *of the blood plasma (Table 53), all cholesterol of the gastro-intestinal* tract is absorbed by the lymphatics. In the rat, about 50% of the cholesterol is present in the lymph in an esterified state (Chaikoff et al. 1952).

It was assumed that the lymphatic apparatus is of some importance *also in the intestinal absorption and transport of iron. McCallum* (1891, 1894) observed already at the end of the last century that, after *the peroral administration of iron, leukocytes were histologically de*monstrable in the intestinal villi which contained iron. Up to most *recent times the conception prevailed that iron absorbed from the in*testines was transported by mononuclear phagocytes and the lymph *vessels (Gilman and Ivy 1947). In contradistinction to the experi*ments carried out with antiquated histological methods, Endicott and his associates (1949) demonstrated in experiments with radioactive iron that neither the iron found in the mesenteric and cervical lymph nodes nor that contained in the duodenal mucosa can originate from a single administration because their quantity did not change after the peroral ingestion of iron; its accumulation takes weeks and months, and it behaves not as transported but as stored iron. Perorally introduced iron was comparatively quickly absorbed in the dog into the portal vein and not by the lymphatics. Gabrio and Salomon (1950) studied the ferritin content in the small intestine and the lymph nodes of horses and found it to have markedly increased 24 to 48 hours after the ingestion of iron. They concluded that ferritin played a role in iron absorption and that the ferritin was transported by the lymph vessels.

As a result of the researches of Peterson and Mann (1952) and also those of Everett, Garrett and Simonds (1954) it was definitively proved that the lymphatics did not participate in the removal of iron absorbed in the *gastro-intestinal tract Peterson and Mann administered*

to rats radioactive iron perorally, subcutaneously, intramuscularly and intravenously. Not more than 0·03 to 0·18 per cent of 1 to 20 mg of perorally administered iron was removed through the lymphatics within 8 hours. At the same time, 70 per cent of the iron appeared in the liver to which it must have gained access via the portal vein. Of the iron administered parenterally, about the same (or greater) quantities were emptied through the intestinal lymphatics. Hence perorally-administered iron that has passed into the intestinal lymph does not, therefore, originate from the intestines but from the blood plasma. These data were substantiated by the experiments of Everett [illegible]

into the blood capillaries, whereas iron attached to protein is absorbed by the lymphatics. Intravenously injected iron appears very quickly in the lymph, probably adsorbed to protein.

The lymph of the thoracic duct contains, beside dissolved substances, also more or less cellular elements mainly leukocytes, but red blood corpuscles, too. How do these cells get into the lymph?

We have seen that corpuscular elements (India ink, graphite suspension, bacteria, etc.) once they have found their way into the interstitial tissues pass into the lymph, and it is only natural that leukocytes and erythrocytes, after having escaped from the capillaries, can do the same.

However, it is not in this manner that the majority of the cells *found in the lymph get into the lymphatic system.* This is evident from the very fact that the lymph contains only very few red blood corpuscles under normal conditions; the majority of the cells consists of white blood cells and even these are mostly lymphocytes (Rous 1908). Peripheral lymph which has traversed no, or very few lymph nodes contains only sporadic cells, whereas in the lymph of the thoracic duct they are quite numerous. Accordingly, Drinker and Yoffey (1941) *distinguish peripheral, transitory, and central lymph.* Peripheral lymph has not yet flowed through lymph nodes, whereas the transitory lymph had already passed through a few lymph nodes, and has to traverse several more. Central lymph has no more contact with lymphoid tissue before being drained into the large veins. If the cells found in the lymph derived from the blood or if they had entered the lymphatics from the interstitial spaces, there could exist no difference *between peripheral and central lymph.*

The observed difference might be explained by the assumption that a part of the lymph is absorbed in the lymph nodes, so that the number of the cells grows relatively higher. This would mean, however, that a very great amount of lymph (not only water but also protein) has to be absorbed in the lymph nodes into the blood capillaries, which is rather improbable. Besides, the proportion between erythrocytes and leukocytes *in the peripheral lymph is different from that observed*

The cells contained in the peripheral lymph seem in fact to proceed from the interstitial space. At rest, the peripheral lymph contains hardly any cellular elements (Yoffey and Drinker 1939a), but their number increases after movement. In a case of Haynes and Field (1931), for instance, the lymph flowing from the hind leg of a dog, after walking for 10 minutes contained 240 white blood cells per cubic cm. Yoffey and Drinker (1939a) found, in the course of their experiments, an average of 550 leukocytes in the peripheral lymph of dogs (mean value of 16 experiments and 110 countings): of these, 280 were lymphocytes, whereas — at the same time — their blood contained 1320 and the lymph of their thoracic duct 7800 lymphocytes per cubic cm. Essentially similar results were obtained from cats. Hence, peripheral lymph contains more red than white blood cells, and the latter consist mostly of polymorphonuclear leukocytes. This observation is thus reconcilable with the assumption that the cells encountered in the peripheral lymph come from the interstitial spaces and that one is dealing actually with the removal of the cells that have escaped from the blood capillaries.

It is natural that when an injury of the blood capillaries allows a greater number of cellular elements to enter the interstitial space, the cell content of the peripheral lymph will show a corresponding increase. Since the majority of the extravasated cells consists of erythrocytes, the lymph is seen in these cases to be bloodstained. A comparatively slight trauma is sufficient to cause such capillary damage. We have repeatedly seen the lymph become markedly bloodstained in cases of anoxia, in ischaemic shock or under the effect of histamine. However, as we have already pointed out, no such serious interventions are necessary: we saw the lymph of the thoracic duct of dogs become distinctly blood-stained after the intravenous injection of 1 ml of novurit.

Of great interest are the experiments of Ross, Furth and Bigelow (1952); they found that the effect of ionizing radiation on dogs and rats was to increase the erythrocyte content of the lymph very considerably. The maximum effect manifests itself two weeks after the irradiation, when the number of erythrocytes in the lymph reaches the two-million mark. This is attributed by the authors to the increased fragility of the capillaries caused by irradiation which has the consequence that a great number of erythrocytes extravasates into the interstitial space. They assume that this phenomenon contributes to the development of anaemia occurring after irradiation. Irradiation is known to induce lymphocytopenia and to reduce also the number of lymphocytes in the lymph.

While peripheral lymph contains relatively few cellular elements, the majority of which consists of red blood corpuscles, things are different in respect of transitory and central lymph which has already passed through lymph nodes.

Baker (1932/33) investigated the number of white blood cells in the mesenteric lymph. He states that the lymph contains 730 (0—2500)

white blood cells before passing through Peyer's patches, and 6800 (300—97 000) after having traversed them, whereas the chyle which has already passed through the lymph nodes, contains 41 200 (1700—143 800) leukocytes per cubic mm.

Tables — compiled from the data of various authors — in the monograph of Drinker and Field (1933) ...ent figures concerning the leuko- ...m different regions. They com- ... of the thoracic duct. According ... lymph of the thoracic duct contains in the dog about 10 000 lymphocytes per cubic mm. Th...

...and found, on an average, ...lymphocyte content of the lymph in rats thoracic duct and 21 500 lymphocytes in the lymph of the thoracic duct and 7065 in that of the cervical trunk.

All these ex... see Drinker a... dence to show ... present in the lymph are principally lymphocytes, and that they cannot originate either from the blood or the interstitial spaces but are formed in lymph nodes whence they pass into the lymph.

A large number of lymphocytes enters the blood circulation through the thoracic duct every day. According to Yoffey (1935/36), in a dog of 10 kg body weight, the number of lymphocytes passing into the blood through the thoracic duct amounts to 212 (30—875) millions per hour and 5076 millions (average of 21 dogs) per day; these figures do not include the lymphocytes that reach the blood path through other channels (e.g. the right lymphatic trunk and other lymphaticovenous anastomoses). Drinker and Yoffey estimate the average number of lymphocytes contained in the blood of a dog of 10 kg body weight at 2500 millions.

Since the number of lymphocytes in the blood is more or less constant, i.e. the number of lymphocytes getting into and disappearing from the blood must be more or less equal, all lymphocytes of the blood must be exchanged at least twice a day which means that the average life-span of the lymphocytes is 12 hours (Ehrlich 1945/46; Adams et al. 1945).

This assumption is supported by experiments in which it was observed that a fistula or the obstruction of th... a prompt ... phoc... bloo... multaneous ... (Biedl and ... The numbe... 24 hours. Th... ...two factors: either not all communica-

tions between blood and lymphatic system are interrupted or there are also other ways in which lymphocytes may reach the blood stream.

Bierman and his associates (1953) found that, in the majority of the patients suffering from lymphoid leukaemia, the lymphocyte content of the lymph of the thoracic duct was constantly less than that of the venous blood and that, on the other hand, the existence of a lymphatic fistula did not reduce the number of lymphocytes in the blood even after 48 hours. These observations may perhaps be explained by the assumption that, in cases of lymphoid leukaemia, the lymphocytes remain longer in the circulation than under normal conditions. The "normal life span" of leukocytes (with an average of 12 hours in dogs and 8 hours in man) does not necessarily mean that they are destroyed after the lapse of this period but only that they disap- [illegible] intestinal lumen through the intestinal mucosa.

The number of lymphocytes carried into the circulation by the lymph did not exceed the normal daily figure of 3500 millions in the leukaemic patients of Bierman and his co-workers.

The lymphocyte count in the blood was, however, very high (133 to 562 thousand millions), from which the authors concluded that it was not mainly via the thoracic duct that the lymphocytes reached the circulation. The three patients suffering from lymphoid leukaemia indic[illegible] lym[illegible] into [illegible] mea[illegible] about 17 days; it is, therefore, to be supposed that the thoracic duct is perhaps really not the main path through which the pathological lymphocytes pass into the circulation.

This concept seems to be substantiated by certain as yet unfinished experiments (Szabó, Rév and Pályi): having transfused the blood of patients with lymphoid leukaemia into non-leukaemic persons (incurable cases of carcinoma) we found that the transfused lymphocytes disappeared from the circulation after a few hours.

The life of the lymphocytes is therefore not abnormally prolonged, [illegible] leukaemia are thus only explainable by the assumption that the

number of leukocytes entering the circulation is considerably higher than that in normal persons; since Bierman and his co-workers found that the number of lymphocytes which passes into the circulation through the thoracic duct is not more than in normal individuals, it seems evident that in these cases the greater part of the lymphocytes *does not reach the circulation via the lymph vessels.*

Many authors investigated those conditions in which the number of the lymphocytes circulating in the blood is changed and lymphocytosis or lymphopenia brought about.

As has been noted, pilocarpine causes lymphocytosis, an increase in the number of lymphocytes in the thoracic duct lymph (Rous 1908). According to Harvey (1906/07), lymphocytosis brought about by pilocarpine is due to the contraction of the smooth muscle fibres in the lymph nodes and the spleen. It forces the lymphocytes out of the lymphoid organs. The effect of pilocarpine, the mechanism involved being a neurogenous one, appears very quickly. However, various other factors are also known to induce lymphocytosis: its course is slower in these cases and the mechanism a different one.

Earlier investigations have proved that foreign proteins, heat and cold, X-rays, etc., may also cause lymphocytosis which develops more slowly, lasts longer and is accompanied by the hyperplasia of the lymph nodes and the lymphoid tissues all over the body (Wiseman 1931; Nakahara and Murphy 1921), etc. More recent investigations do not, however, substantiate these data. It is nowadays universally recognized that the production of lymphocytes is regulated by the adrenal cortex and that the number of circulating lymphocytes is inversely related to its activity (Dougherty and White 1947); Valentine et al. 1948; Yoffey 1950). Accordingly, any dysfunction of the adrenal cortex (Addison's disease, adrenalectomy) induces lymphocytosis (Heidinger 1907; Zwemer and Lyons 1928), while adrenocortical hormone, i.e. glucocorticoid (Dougherty and White 1944), or a stimulation of adrenocortical secretion causes lymphopenia. A stimulation of the adrenocortical secretion provokes lymphopenia irrespective of whether it was effected by the injection of adrenocorticotropic hormone or by different "stresses", e.g. cold, starvation, loss of blood, excitement, electric shock, X-rays, histamine, adrenaline, etc. (Gellhorn and Frank 1948, 1949; Luft et al. 1950; Graham and Cleghorn 1952; Dougherty and White 1946).

Hungerford and Reinhardt (1948) showed that the number of [illegible] of the thoracic duct in [illegible] ctomy likewise causes [illegible] 1952a, b). Reinhardt [illegible] and Baxter (1946) in [illegible] lymph of the thoracic [illegible]. On the other hand, growth hormone, pitressin, pitocin, adrenocortical extract, cortisone acetate and desoxycorticosterone are regarded by the above authors as

agents that do not affect the number of leukocytes, but admit in a recent *publication that the number of lymphocytes contained in the* lymph of the thoracic duct is considerably decreased by cortisone and by hydrocortisone in particular.

But all these investigations failed to elucidate the question whether lymphopenia induced by the stimulation of the adrenal cortex is brought about through the destruction of the lymphocytes circulating in the blood (and in the lymph) or through a decrease in the production of lymphocytes. This question is justified for the following reason: while it was demonstrated by Hechter and Johnson (1949) *as well as* Hechter and Stone (1949) that adrenocortical hormone alone did not increase the destruction, i.e. the lysis of lymphocytes *in vitro* the addition of adrenocortical extract nevertheless increased the lysis of lymphocytes in the blood perfusing the isolated spleen, as also in blood *incubated with lymph-node homogenate. Adrenocortical hormone* induces the acute involution of lymphatic tissues, e.g. lymph nodes, thymus, etc. (Wells and Kendall 1940; Dougherty and White 1945). The essence of this action consists in the destruction of lymphocytes in these tissues. Therefore, theadrenocortical hormone probably regulates the number *of the lymphocytes in the circulating blood by the* degeneration of the lymphocytes contained in the lymphatic tissues so that the number of lymphocytes reaching the blood via the thoracic duct becomes less; it is probable that also other mechanism are involved. It is thus clear that, according to these experimental data, *there is no lymphocytolysis in the circulating blood itself.*

We have already pointed to the fact that the number of lymphocytes in the lymph is influenced not only by the adrenocortical hormones but by adrenaline as well. It has long been known that adrenaline induces lymphocytosis (Garrey and Bryan 1935). However, more recent *investigations have proved that this lymphocytosis is only of a temporary* nature and lasts only a very short time. According to Luft and his associates, lymphocytosis (and leukocytosis) exists only during the injection of adrenaline, and the number of lymphocytes then gradually drops below the original value. The initial increase used to be attributed to the *contraction* of the spleen, since, however, the phenomenon has been observed also in splenectomized animals, it seems more likely that it is due to the contraction *of the* smooth muscle fibres of the lymph nodes (Martin 1932). The cause of subsequent lymphopenia is, however, the stimulation of the hypophyseal-adrenocortical system, and it fails to occur in adrenalectomized animals (Gellhorn and Frank 1948).

*It has already been mentioned that the lymph nodes probably* play a very important part in the production of immune bodies. More recent data support the assumption that immune bodies, as also the entire $\gamma$ globulin fraction of the plasma proteins, are produced in the lymphocytes themselves and become free at their lysis. The factors which increase lymphocytolysis (adrenocortical *hormone, stimulation*

of the adrenocortical function) also promote the formation of immune bodies and the augmentation of the $\gamma$-globulin fraction of serum proteins (White and Dougherty 1945; Cradock et al. 1949, etc.). On this question and, generally, on the origin and destruction of lymphocytes an almost countless number of reports have appeared. But all this exceeds the scope of the present work and has only been mentioned for the sake of completeness.

# CHAPTER XII

# INSUFFICIENCY OF LYMPH CIRCULATION

The physiologists of the past century still considered oedema as a problem of lymph circulation; after Starling's works, however, it seemed that the disturbances of the water balance could be sufficiently explained even with a complete disregard of the lymph circulation. A good many authors had actually as good as forgotten the existence of the lymphatic system.

We are convinced that every oedema is — beside other factors — also indicative of insufficient lymph circulation. Insufficiency of lymph circulation is interpreted by us, in the sense of Korányi's functional pathology, to mean that the lymph vessels become unable to comply fully with the task of continuously draining the interstitial spaces. Of course, we do not suggest that oedema is always a consequence of a primary disturbance of the lymph circulation as was assumed before Starling; however, such cases are also known to occur (lymphoedema). Insufficiency of the lymph circulation is mostly a secondary phenomenon; it means that, for some reason, the lymph circulation cannot compensate for the consequences of increased capillary filtration. From this point of view the cause of increased filtration, i. e. the factor responsible for the upset of Starling's equilibrium is indifferent: it makes no difference whether it is an increase of capillary pressure, a decrease of colloid-osmotic pressure caused by hypoproteinaemia or a diminution of effective colloid-osmotic pressure caused by the increased permeability of blood capillaries; the appearance of oedema invariably indicates that the lymphatics have failed to remove all the fluid from the interstitial spaces

Let us examine e.g. the pathogenesis of the two most important types, cardiac and nephrotic oedema. In patients with generalized phlebohypertension, increase of capillary pressure leads to augmented capillary filtration. Increase in capillary pressure signifies *"disposition to oedema"* (*"Ödembereitschaft"*) stagnant — and to a smaller extent arterial — hypoxia starts, as demonstrated by Földi (1954), regulative mechanisms of a central origin which lead — partly together with haemodynamic changes and partly independently of them — to a retention of sodium in which the direct effect of renal phlebohypertension also plays a certain part. We are, therefore, faced with a simultaneous susceptibility to oedema and a retention of sodium (and fluid); however, *oedema does not arise unless the lymphatic system is incapable of draining the increased amount of interstitial fluid.* It has already been mentioned that a disturbance of the lymph circulation is actually demonstrable in cases of phlebohypertension: the increase of venous pres-

sure reacts on the lymphatic system, intralymphatic congestion arises which leads to the development of a mechanical-functional insufficiency of the lymph flow.

The situation is similar in the case of nephrotic oedema. Glomerulitis, the increased permeability of the glomerular membrane, is here the primary lesion which leads to proteinuria and hypalbuminaemia, in addition a mechanism not yet known in all its details, probably of a regulative nature, leads to the retention of sodium and fluids. An increased amount of fluid will therefore stream from the blood capillaries into the interstitial spaces, but it is clear that *as long as the lymph channels are able to carry off the filtered fluid, oedema cannot arise. The appearance of oedema indicates in this case also an insufficiency of the lymph circulation*, although by means of a mechanism different from that involved in cardiac oedema. We have seen that in such cases there is an immense fluid flow in the lymphatic system; only when even the maximum lymph flow becomes relatively insufficient, does oedema arise. It is in such cases that we speak of a dynamic or relative insufficiency of the lymph flow.

In cases of any generalized or local oedema the situation is also — *mutatis mutandis* — the same as in phlebohypertonic and hypalbuminaemic oedema. It was for this reason that in the investigation of the pathogenesis of every type of oedema we adopted the working routine of asking: *of which type is the insufficiency of lymphatic circulation?* We shall see in the following chapters numerous examples of this method, and we shall also see that it has helped us in explaining a number of pathological conditions on a uniform basis.

The results of our investigations suggest the distinction of the following types of lymph-circulatory insufficiency:

I. *Mechanical insufficiency*
   1. Organic (anatomical causes)
      a) Occlusion of lymphatics
      b) Extirpation of lymphatics or lymph nodes
   2. Functional
      a) "Haemodynamic insufficiency"
      b) Lymphangiospasm
      c) "Akinetic insufficiency"
      d) "Valvular insufficiency"

II. *Dynamic insufficiency*

III. *Insufficiency of absorption*
   1. Change of proteins?
   2. Change of interstitial space?
   3. Change of lymph capillaries?

I. *Mechanical insufficiency* is present, where lymph flow is hindered by a mechanical factor. The mechanical factor may be of an anatomical, organic nature, e.g. the occlusion of lymphatics by obstructive lymphangitis, thrombosis of the lymphatics, filariasis, "lymphangitis cancerosa", etc. As a good example of obstructive lymphangitis, let us refer once more to Drinker's experiment in which he produced an obliteration of the lymph vessels by means of quinine silicate injections, or let us mention the occlusion of human lymphatics in pneumoconiosis.

We observed lymphatic thrombosis in the course of our experiments, e. g. in experimental cholangitis. A radical extirpation of the regional lymph nodes produces — as we shall see — oedema in certain cases, especially if the operation is accompanied by a sclerotic atrophy of the tissues as a consequence of irradiation.

The mechanical insufficiency of lymph circulation may also be of a *functional nature. It has already been noted that if pressure increases* in the large venous trunks, such increase may react upon the lymphatic system; *we have seen the role of this mechanism in the haemodynamic* insufficiency of lymph circulation, in the pathogenesis of phlebohypertonic oedema. We have also seen that lymphangiospasm can be produced in animals by a stimulation of the sympathetic nerve fibres; we believe that we are justified in ascribing a role to lymphangiospasm in human pathology whenever oedema (e.g. thrombophlebitic oedema) can be impeded by sympathectomy or procaine infiltration, but also when a stimulation of the nervous system seems to be present, e.g. in inflammation or in other pathological conditions.

We have seen that in the region of the extremities lymph flow is maintained by muscular activity and we emphasized the decisive role of the lymphatic valves in this mechanism. If, for some reason, the lymph vessels dilate so that the valves cease to operate, it will produce a "valvular" insufficiency of the lymph circulation. This may be seen in some forms of elephantiasis. If, however, muscular contractions cease completely, lymphatic transportat is abolished ("akinetic insufficiency"). This is, for example, one of the main causes of oedema in cases *of causalgia and paralysis. It is by this mechanism that* Exton-Smith and Crockett (1957) explain oedema occurring in paralysed extremities.

II. To the other main form of the insufficiency of lymph circulation we have applied the term *dynamic insufficiency.* We have seen that when capillary filtration is increased, e.g. on the active movement of an extremity, also after the scalding of an area, etc. lymph flow becomes more copious. If the augmentation of lymph flow can keep pace with the increase in capillary filtration, oedema does not arise. The transport capacity of the lymph ducts is, however, limited: if the supply of fluid is raised, the amount of lymph removed during time unit will rise for a certain time, but sooner or later it must reach a maximum when the transport of still more fluid becomes impossible

at a given cross-section and pressure. A disproportion between filtration and absorption will arise, so that a decompensation of the intermediary water balance, oedema, is established. It is in these cases that we speak of dynamic insufficiency. Examples hereof have already been quoted in connection with hypalbuminaemic oedema, and it will often be encountered in the special part of this book. In the discussion of serous inflammations we shall, for instance, see that an increase in the permeability of blood capillaries, the outflow of a great amount of protein-rich fluid into the interstitial space is accompanied by the known histological signs of serous inflammation only if the lymphatic system is no longer able to keep the tissues "dry".

III. Finally, we have recognized also a third form of the insufficiency of lymph circulation: *the absorptive insufficiency* of the lymph flow. In this case — owing to a change in the protein or a change of the interstitial space itself, and sometimes a change in the wall of the lymph capillaries — protein-rich fluid will accumulate in the interstitial space. We are thinking here in the first place of structural changes, a possible change in the electrical charge of the colloids, the precipitation of pathological proteins or their binding to the ground substance of the connective tissue. The notion of the insufficiency of absorption contains still a good many hypothetical elements; nevertheless, the facts quoted in the preceding chapters which show that the diffusion of colloids in the connective tissues and their penetration into the lymphatics may be influenced by ferment poisons, further, that diffusion — approach to the lymph capillaries — is dependent to a large extent on conditions of adsorption; that the endothelial cells of lymph capillaries take up protein; that the permeability of the lymph-capillary walls may be influenced by hyaluronidase, etc., seem to justify the introduction of this notion. Pathology also compels this [illegible] ...ated, [illegible] im- [illegible] tion [illegible] can be assumed. Let us refer here to the observation made by us in cases of struma (see the corresponding chapter) where we succeeded in demonstrating changes of a degenerative character in the endothelial cells of the lymph capillaries.

With regard to the late consequences of the pathological process, distinction must be made among the different forms of the insufficiency of lymph circulation. In the acute phase, any form of the insufficiency provokes oedema. It is, however, known, that accumulation of protein-rich fluid in the interstitial space leads to the formation of new connective tissue, then to fibrosis followed by cicatrization and sclerosis, which give rise to anatomical changes in the affected area that entail grave functional consequences. We think that in the pathogenesis of all these cases a certain form of the insufficiency of lymph circulation has a decisive importance; if lymphatic drainage is sufficient, no oedema fluid will remain in the interstitial space, and no

protein can accumulate. This question will emerge repeatedly in the following chapters in connection with the pathological changes of different parenchymatous organs.

The new pathological notion of the insufficiency of lymph circulation, the distinction of various forms of insufficiency, has been found very useful especially in the investigation of different pathological processes. We must, however, emphasize — and we shall have to point it out again and again — that the different types of insufficiency do not, as a rule, occur in a "pure" form: it frequently happens that various forms of insufficiency are present in combination, thereby complicating and aggravating the clinical picture.

Our investigations (Földi, Papp, Solti and Koltay 1957) show that a generalized insufficiency of the lymph circulation changes the salt and water balance of the organism in the same way as do the other forms of generalized oedema, e.g. of cardiac or renal origin.

The first step of our experiment was a *preparatory operation* in which, by means of right-side thoracotomy, we ligated the right lymphatic trunk, the lymph vessels running in the loose connective tissue of the mediastinum, as well as the azygous vein. This intervention in itself caused, at best, a transitory congestion of lymph extending only to insignificant areas of the body, but it enabled us to stop suddenly all lymph circulation in the course of the main operation by ligating the thoracic duct after local anaesthetization of the neck.

We waited two weeks after the *preparatory operation;* we then ascertained how much of the ingested salt and water was discharged by the animals during a definite length of time (Table 61).

TABLE 61

| | | Control – operation | | | Control – Sham operation | | | Sham operation — operation | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Secreted | Day | $\bar{x}$ | s | p | $\bar{x}$ | s | p | $\bar{x}$ | s | p |
| Na mg | (1) | 993 7 | 136 8 | 0 1 | 354 7 | 324 1 | 10 | 639 0 | 266 6 | 2 |
| | (2) | 392 7 | 197 7 | 5 | 305 1 | 341 0 | 20 | 153 1 | 420 2 | 50 |
| Secreted | (1) | 278 7 | 97 6 | 1 | 101 2 | 153 7 | 30 | 177 2 | 73 2 | 2 |
| water ml | (2) | 133 9 | 39 6 | 1 | 118 8 | 170 8 | 30 | 25 0 | 168 6 | 80 |

This examination was repeated after the lapse of some days following a *sham operation:* under local anaesthesia, we made an incision of 5 cm on the right side of the neck, above the external jugular vein and parallel to it. We isolated and lifted up the carotid artery, and seized the pleural cone a few times with forceps.

After a further interval of 10 days, we ligated the thoracic duct in the neck, as mentioned, under local anaesthesia, two days (later,

four days) after this surgical intervention, we examined the sodium and water metabolism of the animals anew.

By this arrangement is was possible to use each animal as its own control.

We used Student's method in the statistical evaluation of our results.

It can be seen from the table that generalized lymphoedema, the general mechanical insufficiency of lymph circulation, leads to a considerable retention of sodium and fluid. This phenomenon is surely due to the following: when the thoracic duct of our animals is tied off, all inflow of lymph into the blood stream is suddenly stopped; since capillary filtration goes on uninterruptedly, the volume of circulating plasma must temporarily diminish. What essentially happens is an overthrow of Starling's equilibrum: a "dehydration reaction" (Földi 1953; 1954a) is brought about, like that occurring in *plasmapheresis or generalized phlebohypertonia* which must disturb the salt and water balance of the organism.

THIRD PART

# SPECIAL PHYSIOLOGY AND PATHOLOGY OF THE LYMPHATIC SYSTEM

# CHAPTER XIII

# THE HEART

## INSUFFICIENCY OF LYMPH CIRCULATION IN THE HEART

Lymph flow from the lymphatics of the heart is continuous and of a comparatively considerable volume. Drinker and his associates (1940) tied a cannula into the efferent cardiac lymphatics of dogs and collected 5·2 to 27·6 mg of lymph per minute from animals of an average body weight of 10 kg. In these experiments, Drinker and *his co-workers drew lymph only from one of the efferent main lymph* trunks because it was assumed that the heart of the dog contained only a single efferent lymph trunk. In the course of our experiments we succeeded in showing that the heart of the dog also had, as a rule, two efferent main lymph trunks, so that the figures of Drinker must *be lower than the real ones. It may safely be assumed that the amount* of lymph flowing from the heart of dogs per minute is about twice as *much as that indicated by Drinker and his associates.*

That the heart is provided with a wide network of lymph vessels has already been mentioned in the anatomical chapter. It has also been mentioned that the lymph flow in the cardiac lymph channels is considerable. The question as to what happens when cardiac lymph circulation becomes insufficient is therefore justified.

In our experiments (Földi, Romhányi, Rusznyák, Solti and Szabó 1954a, b) we induced a *mechanical insufficiency* by ligating the efferent cardiac lymphatics.

In *preliminary experiments*, we gathered information about the position of the cardiac lymph trunks and regional lymph nodes.

With this in view, we performed thoracotomy in dogs. After opening the right side of the thorax in the 3rd intercostal space, we ligated the mammary arteries and veins, then made a transverse cut across the sternum; this done, we extended the incision to the left 3rd intercostal space, thus laying open a large area. Then we opened the pericardium and injected India ink into the myocardium at different points

The *India ink injected into the myocardium became visible in* the fine lymphatics within a few seconds, and it could be observed how it was propelled by every contraction of the heart; soon also the efferent extracardial lymphatics became visible. In the course of these experiments it became quite clear that — in contradiction to Drinker *and his associates — the lymph of the right and left half of* the heart in the dog is drained by two separate lymph trunks, the same as in human subjects. These lymph trunks empty then into one or two lymph nodes. As a rule a lymph node is encountered between and

above the superior Vena cava and the aortic arch; sometimes there is another lymph node cranially from and to the left of this point. As in other regions of the organism, the course of the lymph ducts displays a great variability in the heart also: certain vessels travel towards other lymph nodes, while others are in communication with the thoracic duct or the right lymphatic trunk.

In order to obtain a possibly complete mechanical insufficiency of the cardiac lymph circulation, we tied off in our *main experiments* not only the two lymph nodes but also the thoracic duct, the right lymphatic trunk and the retrosternal lymph vessels. By this procedure we produced as "complete" a lymph congestion as was obtainable within the limits of anatomical possibilities, although we are not absolutely sure that we really succeeded in tying off all efferent lymphatics of the heart.

It should be emphasized that the pericardium was not opened, not even touched, in our main experiments so that the surgical interventions were never followed by sterile pericarditis; of this we made sure in each case by macroscopic and later by microscopic examinations. It seems to us important to point to this fact, since pericarditis is known to give rise to certain changes in the electrocardiogram.

Another technical detail to which we want to point is that we took great care not to damage or interrupt any nerves during the intervention. Special attention was paid also to this question in the pathological-anatomical examination. The lymph vessels which run to the lymph nodes and had been hardly perceptible before, became dilated by congested lymph after the ligation of the cardiac lymph nodes, and the stringlike arrangement of the valves in the lymphatics became clearly visible.

We took electrocardiograms of the animals before and immediately after the operation, then again some hours later, and daily or every two days thereafter. The dogs were killed by intravenous evipan injection at various dates after the operation; the heart was then fixed in hot formalin for histological examination. The earliest time at which we sacrificed the animals was 30 minutes after the operation, and the longest period of observation lasted two weeks.

We made, of course, control experiments also by way of sham operations in which we opened and closed the thoracic cavity of dogs, and made electrocardiograms. None of the electrocardiographic and histological alterations to be described in the following was observable in these control animals.

Our experimental results revealed striking and characteristic changes. As a rule, they did not manifest themselves immediately following the intervention but developed gradually, and it was 2 to 5 days after the operation that they were most marked after which they gradually disappeared in the majority of the cases.

The observed ECG-changes may, on the whole, be divided into three principal groups. The picture observed in the first group re-

sembles that usually seen in *myocardial hypoxaemia:* the *ST* section is markedly depressed and the *T* waves are flattened or even become negative. Twelve of the twenty-two examined dogs belonged to this group. The extent of the changes, was of course, different in each case. In some cases, the negativity of the *T* waves was not striking, while pointed, deeply negative *T* waves ("coronary *T*"), marked *ST* depression, sometimes a deepening of the *Q* waves were observed in all the three leads in other cases.

The second group includes the cases where in all three leads marked elevation of the *ST* section, pointed negative *T* waves and frequently deep *Q* waves (Pardee's *Q*) were observed. The picture resembles the electrocardiogram usually observed in cases of infarcerations of the anterior and posterior wall (Katz's type). To this group belonged four of our cases.

In five cases we saw a very marked elevation of the *T* waves, especially in the second and third leads. The picture corresponds to the "Anoxia—*T*" described in grave anoxaemia.

It was only in a single case that no essential ECG change could be seen.

*In addition to the typical changes included into the three groups we saw in some cases disturbances of impulse formation and conduction. Atrioventricular block was observed in two cases* and ventricular extrasystoles in *one* (Figs. 182—184).

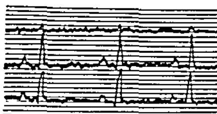
No precise correlation between histological picture and electrocardiogram seemed to exist. We saw in four cases *interstitial oedema of the myocardium* (Figs. 185—186) with dilated lymph vessels and *local interstitial infiltrations, the latter consisting of lymphoid cells and an increased number of connective-tissue cells.*

In three cases, still graver lesions were encountered in the myocardium, namely *disseminated focal necroses,* especially in the wall of the right ventricle (Figs. 187—189). In five cases, only dilated veins, capillaries and lymphatics were found in the heart muscle, while the histological picture was negative in five cases.

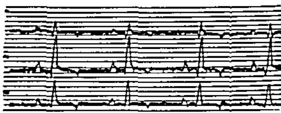
It is quite evident that the ECG revealed grave symptoms in all those cases where histological examination indicated the presence of myocardial necroses, but we had also cases in which the histological picture was negative in spite of pronounced electrocardiographic changes.

One of the reasons why there is no close relationship between the histological pictures and the ECG records is surely *the fact that we sacrificed the animals at a time when the electrocardiographic changes were already in regression,* viz. usually *more* than five or six days after the occlusion of the lymph channels; we think it very likely that, at the climax of the electrocardiographic changes, we should have found histological alterations also in those cases which revealed later negative histological pictures. The other cause of incongruity may be the focal nature of the changes and perhaps

*Before operation*



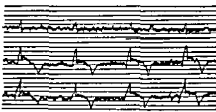
*One day after operation*



*2 days after operation*



*3 days after operation*



*4 days after operation*



*Fig. 182.* Electrocardiographic changes after ligation of cardiac lymph nodes

*Before operation*



*Immediately after operation*



*On the day after operation*



*3 days after operation*

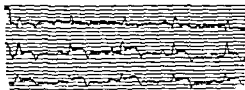


'ig. 183. Electrocardiographic changes after ligation of cardiac lymph nodes

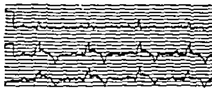
*Before operation*



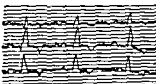
*On the day after operation*



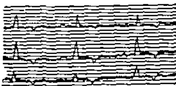
*2 days after operation*



*3 days after operation*



*4 days after operation*



*5 days after operation*

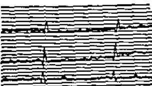


*Fig. 184.* Electrocardiographic changes after ligation of cardiac lymph nodes

also our procedure that we made no serial sections but excised only single pieces of the myocardium.

How are we to explain the regression of the electrocardiographic changes? It is, we think, beyond any doubt that the occlusion of the



*Fig. 185.* Lymphoedema in cardiac musculature. Interstitial oedema A dilated lymph capillary, lined with endothelium



*Fig 186.* Lymphoedema in the heart A dilated lymphatic with valve is visible

lymph circulation is never complete; further, the strong regenerative power of the lymphatics must also be taken into consideration. Notwithstanding the ligation of the cardiac lymph nodes as well as of the

already [illegible]
of our n[illegible]
pericard[illegible]
to pericarditis. [illegible] any histological symptoms pointing

In some cases we saw especially high $T$ waves, usually in connection with the deformation of the $ST$ section; this occurs, as Dietrich (1924) suggests, in cases where the supply of the myocardium with oxygen is strongly decreased as in grave hypoxaemia. In our experiments we ascertained, therefore, the degree of oxygen saturation in the arterial blood and found it to be normal so that the "Anoxia —$T$" must have been due in our cases not to general but to *local* anoxia.

As regards now the application of these observations to human pathology, the following can be said:

It is known that, in a part of the patients suffering from carditis, the ECG curves resemble those obtained in the gravest cases of coronary insufficiency (Lepeschkin 1951; Holzmann 1953). We also saw more than one case of carditis where — though infarceration could be excluded with certainty — ECG changes suggestive of myocardial infarceration were observed. If we take into consideration Primak's (1940) report who observed dilated lymph vessels and lymph congestion in the myocardium of patients, who died of carditis, and if we also consider Szutrély's statement (personal communication) that carditis in children is usually fatal if associated with mediastinopericarditis and that autopsy in such cases reveals the existence of myocardial oedema, the conclusion that, *in carditis, interstitial oedema and dilation of the lymphatics indicate the insufficiency of cardiac lymph circulation* seems to be obvious. Only the careful histopathological examination of a large material could tell whether this insufficiency is of a dynamic nature — serous inflammation, increased permeability of blood capillaries and exudation exceeding the transport capacity of the lymphatics — or of a mechanical character (e.g. lymphangiospasm or perhaps obstructive lymphangitis and perilymphangitis). That one is dealing with hypoxaemia caused by interstitial oedema in all those cases of carditis where similar ECG curves are obtained that [illegible] ion without the presence of symptoms [illegible] to be an assumption justified by the re[illegible]

## EFFECT OF VENOUS CONGESTION AND SIMULTANEOUS INSUFFICIENCY OF CARDIAC LYMPH CIRCULATION ON THE MYOCARDIUM

Whereas, so far, authors have not concerned themselves with the mechanical insufficiency of the lymph flow in the heart or its consequences, there exist several reports concerning the disturbances of the venous circulation in the myocardium and their histological

and electrocardiographic consequences. It was, for instance, found by Banti (1895, cit. Kunos and Temesvári 1954) that a long lasting venous congestion produced fibrosis in the myocardium which was most pronounced in the musculature of the atria and auricles. According to certain reports, myocardial fibrosis — especially in the right atrium and to a smaller extent in the right ventricle — develops in cases of mitral stenosis. Liebermeister (1922) observed histological changes in cases of myocardial venous congestion caused by relative tricuspidal insufficiency. Condorelli (1931) observed fibrosis in the myocardium of patients who had died of mitral stenosis.

The literature contains reports on the thrombosis of the coronary sinus, e. g. the case of Warner and Dauphinée (1936). Achard, Bariéty and Wilm (1932) observed grave dyspnoea and cyanosis in febrile influenza. At autopsy, they found in the myocardium thrombophlebitis, thrombosis of the coronary sinus and myocardial infarceration.

Laufer (1935) ligated the coronary sinus in animal experiments, and found that the animals survived very long after the intervention, that the musculature of the ventricles had remained practically intact, and that, first, there were signs of venous congestion in the musculature of the atria where fibrosis was seen to have developed subsequently. A similar observation was made also by Condorelli.

Several authors studied the changes produced by a ligature of the coronary sinus in animals. According to the observations of Gross, Silverman and Master (1936), the initial deflection becomes notched and negative, the *ST* section becomes higher, the *T* waves sometimes negative, and transitory irregularities of the heart rhythm occur also. According to Unghváry (1948), a few minutes after the tying off of the coronary sinus low voltage is brought about. The ECG curve becomes normal again very soon after the loosening of the ligature. Unghváry declares that low voltage is just as characteristic of disturbed myocardial venous circulation as is the so-called "infarct ECG" of disturbed arterial circulation.

Recently, Kunos and Temesváry (1954) discussed the histological and electrocardiographical consequences of disturbed myocardial venous circulation.

The electrocardiographic changes observed by them may be divided into three phases. Certain ECG changes manifested themselves already during the operation: they were mainly due to the surgical intervention itself and disappeared after the closure of the thorax. On the days following the operation, the ECG curve resembled that seen in myocardial hypoxia; these changes gradually subsided about 10 days after the operation. Between the 25th and 50th day following the operation a picture of chronic myocardial damage was gradually developing. Histologically, Kunos and Temesváry first saw symptoms of venous congestion which were then followed by changes of a degenerative character but never by myocardial necrosis. The ligature of the coronary sinus had no fatal consequences.

Induced venous congestion is, hence, not followed by grave consequences, presumably because the increased amount of interstitial fluid is carried off by the intact lymphatic system. But we have seen that the occlusion of the lymphatics of the heart causes grave damage even if the blood circulation is unimpaired. Hence, we had to ask ourselves: *what happens if the lymphatics of the heart become obstructed just at a time when they have to fulfil an increased task in maintaining fluid circulation in the myocardium,* i.e. in a case of *venous congestion?*

We investigated, therefore, the processes occurring in the heart, at a time when a simultaneous venous and lymph congestion is induced in the myocardium (*Földi, Romhányi, Rusznyák, Solti, Szabó* and *Temesváry* 1954).

We proceeded in the following manner: after making a normal electrocardio- [illegible] off the coronary [illegible] ct and the [illegible] recorded until the death of the animal.

The experiments gave very interesting results. The most striking *observation was that, although, alone, neither the ligature of the* coronary sinus nor the interruption of cardiac lymph flow represented any serious danger for the animals, the simultaneous tying off of both had the result that 12 of the 13 animals had died between the 3rd hour and the 6th day, i.e. within 41 hours on an average, after the operation, so that only a single animal was still alive at the end of our experiments, i.e. on the 84th day after the operation.

Interesting were the electrocardiographic changes. Whereas — as we have seen — the above-discussed electrocardiographic changes produced by cardiac lymphoedema and, separately, by venous accumulation subsided within a few days, combined ligation produced very grave and progressive ECG changes: low voltage, the typical picture of myocardial infarction, arrhythmia and in some cases "Anoxia—*T*". Intraventricular conduction was disturbed in one of the cases (Figs. 190—193).

In the same way as with the two separate interventions it is the interruption of the cardiac lymph flow that produces a graver ECG picture than the ligation of coronary sinus, so it is the congestion of lymph [illegible] effect of combined ligature [illegible] e made several [illegible] 2). We observe [illegible] but low voltage resulted; then, scarcely a few minutes after the interruption of the lymph flow, the ECG tracing grew rapidly worse: first an elevation of the $ST_1$ appeared, while 10 minutes after the ligature of the cardiac lymph node the low voltage became very pronounced

and — immediately after the operation — the *ST* section in lead II also became elevated.

It is worth while discussing a case separately which survived the ligation of the coronary sinus and the cardiac lymphatics by 3 months.

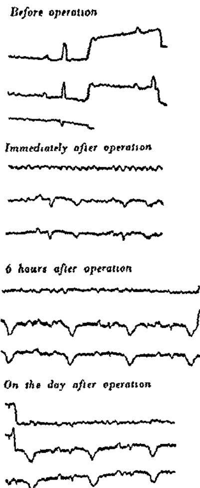
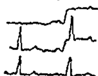



*Fig. 190.* Electrocardiographic changes following ligature of coronary sinus and cardiac lymph nodes

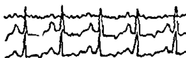
In this animal, a whole series of grave ECG changes could be observed (Fig. 191).

Immediately after the operation, the waves $T_2$ and $T_3$ became pointed and negative, while — on the next day — the $ST_2$ and $ST_3$ became elevated, and the waves $T_2$ and $T_3$ deep and pointed: a

*Before operation*



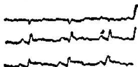
*Immediately after operation*



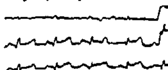
*On the day after operation*



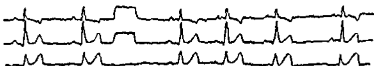
*3 days after operation*



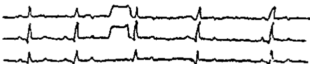
*4 days after operation*



*15 days after operation*



*34 days after operation*



*84 days after operation*

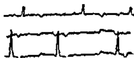
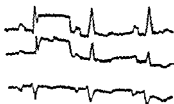
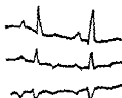


*Fig. 191.* Electrocardiographic changes after ligature of coronary sinus and

*Before operation*



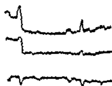
*During operation, after the opening of the thorax*



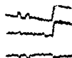
*During operation after the ligation of the coronary sinus*



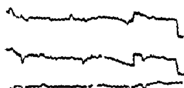
*After ligation of the cardiac lymph vessel*



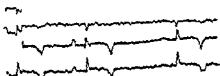
*10 minutes after ligation of the cardiac lymph vessel*



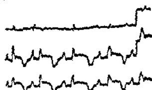
*Immediately after operation*



*6 hours after operation*



*On the day following operation*



*2 days after operation*



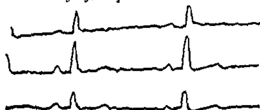
Fig 192 Electrocardiographic changes after ligature of coronary sinus and cardiac lymph nodes

picture of the infarction of the posterior wall had developed. On the third day after the operation, $T_2$ and $T_3$ became pointed, high and positive. On the 15th day following the operation, $T_1$ was negative, the elevation of $ST_2$ and $ST_3$ decreased and the low voltage had ceased. Cardiac arrhythmia developed. On the 35th day after the

*Before operation*



*Immediately after operation*



*On the day after operation*

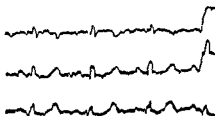


*Fig. 193.* Electrocardiographic changes after ligation of the coronary sinus and cardiac lymph nodes

operation, the elevation of $ST$ had ceased, $T_2$ and $T_3$ had become lower and $T_1$ positive-negative. On the 84th day following the operation $T_2$ was negative, whereas $ST_2$ and $ST_3$ were depressed and showed an oblique downward course.

*The morphological picture* explains both the gravity of the ECG changes and the high rate of mortality. Most of the histologically examined specimens showed even macroscopically extensive haemorrhages in the myocardium, whereas the microscopic picture revealed diffuse haemorrhagic necrosis of the myocardium, interstitial oedema and extremely dilated lymphatics (Figs. 194—199; Table 62). Let us

remember in this connection that the ligation of the coronary sinus in itself produces at this stage only symptoms of venous congestion, and that of the lymphatics only interstitial oedema and rarely focal necrosis. Combined, these interventions lead to grave qualitative changes.

It would as yet be premature to say how our present observations can be applied to human pathology. Our investigations have at any rate made it clear that lymph flow is of primary importance for the heart, and have also made it evident that when a myocardial venous thrombosis appears in human subjects, its consequences will largely depend on the behaviour of the cardiac lymphatic system. It has already been pointed out that the venous thrombosis of the limbs is



*Fig. 19.* Ligation of the coronary sinus and the cardiac lymph nodes. Haemorrhagic necrosis of myocardium

freque [illegible] thrombophlebitis with l [illegible]

It [illegible] on of the venous thrombosis of the heart with the occlusion of the lymphatics is almost invariably fatal. It is possible that this finding may become therapeutically useful in the future when we shall be able to somehow relieve the lymphangiospasm which accompanies the thrombosis of cardiac veins.

Apart from the disturbance of the venous circulation caused by the thrombosis of cardiac veins venous congestions of a haemodynamic origin merit attention. Unghváry (1918) wrote the following in this respect:

"Apart from axial changes, anatomical and functional lesions in the heart musculature also are regarded as factors leading to low voltage. My experiments encourage me to suggest that one should look out for a possible disturbance of the venous circulation whenever one is confronted by low voltages due to myocardial lesions. The most frequently occurring forms of low voltage attributed to myocardial lesions have, so far, been encountered in infarcts and mitral stenoses.

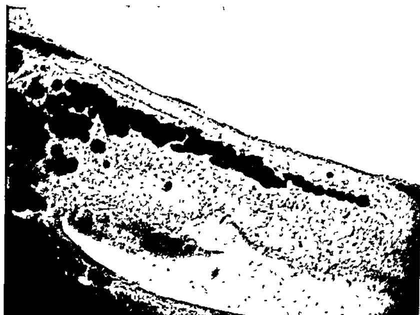


*Fig 195.* Ligation of the coronary sinus and the cardiac lymph nodes. Dilated lymphatic and subepicardial haemorrhage



*Fig 196.* Ligation of the coronary sinus and the cardiac lymph nodes. Exceedingly dilated lymphatic, interstitial oedema

In mitral stenosis. the increase of venous blood pressure and venous congestion is finally transmitted to the right atrium because of disturbed circulation. The congestion existing here is further aggravated by atrial fibrillation. It is probable that, in such cases, the drainage

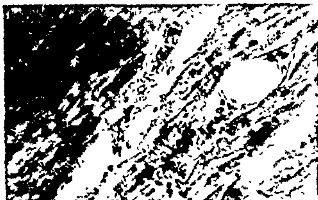


*Fig. 197.* Ligation of the coronary sinus and the cardiac lymph nodes. Blood capillaries surrounded by interstitial oedema, and a dilated, transversally-cut lymph capillary Necrobiosis in cardiac musculature



*Fig 198.* Ligation of the coronary sinus and the cardiac lymph nodes Dilated, blood-filled capillaries, and dilated lymph capillaries in the oedematous intersitial tissue. Signs of advanced necrobiosis visible in myocardium

of the blood of the coronary sinus into the right atrium which is engorged with blood to the point of bursting, becomes difficult if not impossible. Venous congestion, present in the cardiac cavity, induces thus a venous congestion in the heart's own blood circulation. If these arguments are correct, it must be clear from our animal experiments that, under such conditions, the ECG-curve will indicate low voltage. I think the correctness of this statement is supported also by therapeu-

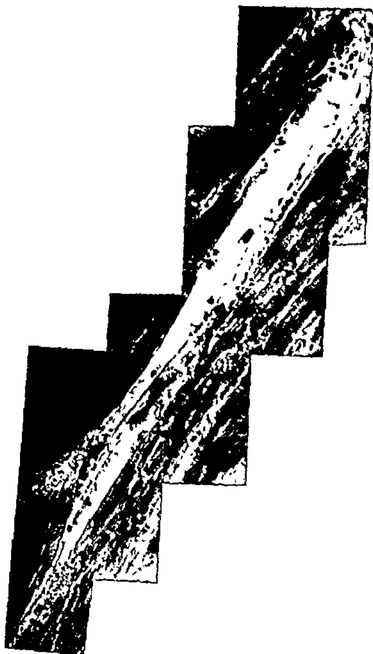


*Fig. 199.* Ligation of the coronary sinus and the cardiac lymph nodes. Oedematous interstitial space. Necrobiotic muscle fibres. The picture shows a longitudinally cut lymph capillary; the endothelial wall of the capillary is easily recognizable

TABLE 62

*Combined ligation of the cardiac lymphatics and of the coronary sinus*

| N° | Survival | ECG | Autopsy |
|---|---|---|---|
| 4299/K 15 | 1 day | Typical picture of an infarct of the posterior wall | Macroscopically haemorrhagic necrosis. Microscopically: extended oedema, enormously dilated lymphatics, subepicardially broad veins, strong haemorrhagic infiltration of tissues with patches of newly-necrotized muscles in them. Leucocytic infiltration round the necrotic muscle fibres. In the musculature of the right ventricle in the oedematous interstitial spaces it is not so much the haemorrhagic as rather a leukocyte infiltration which is predominant. the muscle fibres show increased eosinophil staining, their striation is blurred; sporadically no nuclear staining and traces of beginning disintegration of muscles visible |
| 4301/B 17 | 3 days | Low voltage, then fibrillation $ST_2$-, $ST_3$-elevation and negative $T_1$ | Macroscopically haemorrhagic necrosis. Microscopically: extended haemorrhages in the myocardium. |
| G | 3 hours | — | — |
| 4308/B 18 | 36 hours | Low voltage and disturbed intraventricular conduction, then picture as of an infarct of anterior wall and "Erstikkungs T" | Macroscopically. some light yellow spots in the musculature of the right ventricle visible. Microscopically sporadical subendocardial haemorrhages, no haemorrhage or necrosis in musculature. |
| B 19 | 6 days | Picture of an infarct of the posterior wall | — |
| 282/B 20 | 1 day | $ST_2$-, $ST_3$-elevation, low voltage | Macroscopically spots of the posterior wall of left ventricle filled with blood. Microscopically subpericardially broad, engorged veins, haemorrhages, oedema, partly leucocytic infiltration. Haemorrhages in the musculature of the right ventricle. |
| 5/K 7 | 1 day | Picture of an infarct of the anterior wall | Macroscopically: haemorrhages in pericardium Microscopically. dilated veins No muscular necrosis. |

| Nº | Survival | ECG | Autopsy |
|---|---|---|---|
| K 8 | Still alive after 3 months | See text | — |
| 4296/K 9 | 1 day | Picture of an infarct of the anterior wall | Macroscopically: the superficial, 3—4 mm thick layer of the musculature of both ventricles haemorrhagically infiltrated Microscopically: oedematously thickened pericardium soaked with blood Extended interstitial haemorrhages in the musculature, the muscular fibres between them show eosinophil staining, their nuclear staining is still maintained. In the deeper non-haemorrhagic muscular layer, dilatation of veins, congestion, and oedematous dilatation of interstitial vessels. |
| 4297/K 10 | 1 day | "Flattern" pointed negative $T_1$, $T_2$, $T_3$ | Macroscopically haemorrhages in the musculature of both ventricles, particularly in the anterior wall of right ventricle. Microscopically: extended haemorrhages in the musculature of the anterior wall of right ventricle with disarranged necrotic muscle fibres *Pericardium diffusely, haemorrhagically and oedematously infiltrated* In the musculature of left ventricle, general venous congestion, dilatation of veins, haemorrhagic infiltrations, interstitial oedema, necrosis of musculature. Changes of a multiplex, spotty character, fresh cicatrization *in the left ventricle visible.* |
| K 14 | 3 days | Picture of an infarct of the *posterior wall* | |
| 4300/K 16 | 2 days | Picture of an infarct of the posterior wall | Macroscopically. pericardial haemorrhages. Haemorrhages in subpericardial muscular layer of both ventricles; these extend as far as the lumen of left ventricle The section surface of musculature spotty-haemorrhagically infiltrated. Microscopically: extended haemorrhages with sporadical disintegration of muscles. |
| K 17 | 3 hours | *Picture of an infarct of the* anterior wall | — |

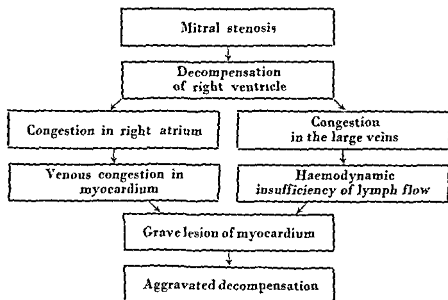
tical results. If we succeed in stopping congestion by means of digitalis, the drainage of the coronary sinus will become normal again so that low voltage will disappear. It can be assumed that in this case the diseased myocardium is not "healed" and the consequent disappearance of the low voltage is not caused by the administration of digitalis during a few days but that the favourable change in the ECG is due to the disappearance of venous congestion".

Relying on the evidence of our own experimental results, we are in a position to modify and continue these arguments as *follows*: as long as the mitral stenosis continues to be compensated, there is increased pressure in the left atrium and in the right ventricle only, *while pressure is still normal in the right atrium, so that blood from* the coronary sinus can flow into the right atrium without hindrance. If, however, the right ventricle becomes dilated and if the consequent increase of pressure extends also to the right atrium, two consequences will immediately appear:

1. the heart's own blood circulation will become more difficult and *myocardial phlebohypertonia* will present itself;

2. as a consequence of *generalized phlebohypertonia*, lymph flow will be impeded in the whole organism and so also in the myocardium and *a haemodynamic insufficiency of the lymph circulation will be brought about.*

Hence, venous and lymph congestion occur simultaneously in the heart musculature which — as already pointed out — must lead to very grave consequences. In this way, the conditions of the heart grows worse by leaps and bounds, and a fatal vicious circle arises:



It goes without saying that the above arguments not only refer to mitral stenosis but — *mutatis mutandis* — to other pathological conditions as well.

## THE LYMPHATICS OF THE ENDOCARDIUM IN CHRONIC ENDOCARDITIS

Little is known about the role played by the lymphatics of the endocardium in the pathogenesis of chronic endocarditis, of the valvular lesions involving hypertrophy. Worth mentioning are, from this point of view, the observations of Eberth and Belajeff (1866): they found no difficulty in injecting the endocardial lymph channels of animals. This was, as a rule, much more difficult in man, especially in regions, showing symptoms of chronic endocarditis. Eberth and Belayeff frequently observed also whitish bundles of a reticular arrangement which — to some extent — resembled obliterated lymphatics. Unfortunately, it is rather difficult to appreciate these findings because of the uncertainty of the methods applied. It would, therefore, be important to renew investigations into this question using better methods.

## CHAPTER XIV

# THE LUNG

*Except water and dissolved crystalloids* — which may be removed also by the blood circulation — *proteins and all foreign substances* which have entered the alveoli or the pulmonary tissue *are removed by the lymphatic system* inasmuch as they are not expelled by coughing. The removal of inflammatory products — plasma proteins, proteins of disintegrating cells, bacteria and bacterial fragments — is, therefore, effected by the lymphatic system in the inflammatory diseases of the lung. The restoration of the anatomical integrity of the pulmonary parenchyma thus depends on the lymphatic system, and *if, for any reason, the pulmonary lymphatic apparatus becomes insufficient so that an accumulation of protein is brought about in the lung, this accumulation will — as we shall see — lead first to oedema and later to fibrosis, sclerosis and pulmonary cirrhosis.*

Cirrhosis of the lung is known to be no independent disease: it may arise as a concomitant of any chronic inflammatory disease of the lung.

## LYMPHATIC SYSTEM OF THE LUNG; PATHOGENESIS OF PULMONARY OEDEMA

Pulmonary oedema is one of the most important and most frequent pathological conditions, and though its clinical picture was described as long ago as 1752 by Malöet, its origin has still not been fully elucidated.

The first fundamental theory of the pathogenesis of pulmonary oedema is that of Welch (1878). In his opinion, pulmonary oedema arises when — *with intact right ventricle* — *the left ventricle becomes insufficient.* Welch tied off the aorta caudally to the origin of the large arteries issuing from the aortic arch which, however, failed to produce pulmonary oedema. It appeared only after ligature of the subclavian and carotid arteries. Welch explained this by assuming that the ligature constricted the vascular path and hampered the functioning of the left ventricle to such an extent as to give rise to pulmonary congestion. Welch's experiment is in harmony with the clinical observation that pulmonary oedema occurs most frequently in those diseases of the heart where either the left ventricle is especially strained — aortic insufficiency, hypertension, coronary sclerosis, coronary thrombosis — or where some other factor provokes a congestion of the lesser circulation (paroxysmal pulmonary oedema of patients with mitral stenosis).

Sahli (1885) induced congestion in the lesser circulation by narrowing the left atrium.

Modrakowski (1914), studying heart-lung preparations, found that, while the increase of perfusion pressure did not give rise to pulmonary oedema, it could be induced by an obstruction of the venous drainage. Pulmonary oedema appeared whenever — with an arterial pressure of at least 35 mm Hg — the venous pressure was so raised as to decrease the difference between the two pressures to 8 mm Hg. When, however, ammonium chloride was added to the blood, pulmonary oedema arose even if pressure values were normal.

Essentially the same was observed by Gorlin, Lewis, Haynes, Spiegl and Dexter (1951) in cardiac patients: whenever pulmonary oedema appeared, the pulmonary capillary pressure was invariably higher than 32 mm Hg. In no case with pulmonary capillary pressure lower than 32 mm Hg could Gorlin and his co-workers observe oedema.

Also a decrease in *colloid-osmotic pressure* may lead to pulmonary oedema. Barry (1928) diluted the blood in heart-lung preparations by means of physiological saline and found that the reduction of the specific gravity of the blood from 1053 to 1050—1045 provoked pulmonary oedema although both arterial and venous pressure had remained unchanged. At a given dilution of blood, the time required for the production of pulmonary oedema is inversely related to

1. arterial resistance,
2. the degree of cardiac insufficiency,
3. the rate of venous inflow.

According to other authors, *pathologically increased capillary permeability* in the lesser circulation leads to pulmonary oedema. It is known that — as was described by Starling himself — pulmonary oedema develops after some time in Starling's heart-lung preparation. The experiments of Lambert and Gremels (1926) proved that this oedema could not be attributed to haemodyn[illegible] [illegible] in the blood used for the purposes of perfusion. It was actually possible to ascertain degenerative changes in the endothelial cells of the blood capillaries by means of histological methods.

Newton (1952) arrived at similar conclusions. This author, too, assumes that pulmonary oedema observed in the heart-lung preparation is due to the toxic substances existing in the blood used for the perfusion. These toxic substances damage the heart itself, as also the bronchial musculature, and influence haemodynamic conditions in the lesser circulation.

It is known from human pathology that pulmonary oedema does not only occur in cardiacs: it appears very frequently after cranial traumata (for details see Herrmann 1951). Pulmonary oedema was frequently observed in connection with intracranial haemorrhage, encephalitis, polioencephalitis, cerebral abscess, cerebral tumour and

meningitis (Weisman 1939; Farber 1937a, b). Gamble and Patton (1951, 1953) induced pulmonary oedema in rats through the lesion of the preoptic nuclei. Pulmonary oedema was further observed after epileptic seizures (Horst 1952).

These data show that pulmonary oedema may also be of a *neural* origin. It is, of course, quite possible, even very likely, that neural pulmonary oedema is — partly at least — due to *a change of circulatory conditions* in the lesser circulation, since the factors releasing neural pulmonary oedema lead, as a rule, to extreme bradycardia, increased blood pressure and decreased cardiac output; it is for this reason that Sarnoff and Sarnoff (1952) as well as Sarnoff and Berglund (1952a) speak of "*neuro-haemodynamic*" pulmonary oedema in connection with pulmonary oedema produced by intracisternal fibrin injection. Gottsegen, Szám and Csornay (1954) succeeded in demonstrating that pulmonary oedema caused by ammonium chloride, which Koenig and Koenig (1949), MacKay et al. (1949), as well as Cameron and Sheikh (1951) assumed to be of a merely neural, adrenergic nature, was likewise of neurohaemodynamic origin. According to Cameron and Sheikh, pulmonary oedema provoked by ammonium [illegible] good results with the use of procaine.

The neurohaemodynamic theory is supported by certain experimental observations. It is, for instance, known that pulmonary oedema can be provoked in rabbits by the intravenous injection of adrenaline. It seems an obvious explanation to attribute this pulmonary oedema to an acute insufficiency of the left ventricle caused by the rapid increase of peripheral resistance. It is, however, stated by Luisada (1940, 1943), Luisada and Sarnoff (1944, 1946), and Glass (1928) that pulmonary oedema caused by adrenaline can be checked by morphine, barbiturate, sympathectomy, the lesion of the quadrigemina, the extirpation of the stellate ganglion and also by a division of the cervical portion of the spinal cord. Luisada suggests that adrenaline causes pulmonary oedema by its action on the central nervous system as well as on the centres regulating the pulmonary circulation and the permeability of the vessels in the lesser circulation.

A further noteworthy observation is that made by Jarisch, Richter and Thoma (1933): pulmonary oedema can be induced in rabbits, rats and guinea-pigs by the intracisternal administration of veratrine and checked by chloral hydrate, urethane or atropine.

Cerebral circulatory disturbances may also lead to pulmonary oedema. Meurers (1925) provoked pulmonary oedema by the ligation of both carotid arteries below the carotid sinus. How difficult it is to interpret experimental results correctly becomes apparent if we think of Welch's above-mentioned experiment in which he ligated the aorta. It is evident that, by so doing, he not only increased peri-

*pheral resistance and retarded the work of the left ventricle but pro*duced cerebral anaemia also.

Also worth mentioning is Luisada's experiment; with the exception of the nerves and the spinal cord, he severed all communications between the head and trunk of dogs and raised then the perfusion pressure in the head: the result was pulmonary oedema. Luisada caused pulmonary oedema in the following manner: with a pressure of 280 to 300 mm Hg, he injected in a short time into both carotid arteries sufficient fluid to double the animal's entire volume of blood. (It should be noted that no pulmonary oedema arose when the same *infusion was given into other arteries*).

Luisada suggests that pulmonary oedema produced in this manner is conditioned by reflexes: he speaks of carotido-pulmonary reflexes arising from the tension of the heart chambers and of the wall of the pulmonary artery: these reflexes give rise to pulmonary oedema if a submaximal amount of fluid is injected which, in itself, would not suffice to bring about oedema. *Denervation of the carotid sinus*, injection of benzodioxane which has a sympatholytic effect, further curare, barbiturate, morphine, chloral hydrate, procaine, atropine + physostigmine have proved to be suitable means of preventing pulmonary oedema.

It is known that pulmonary oedema is produced by the aspiration of various substa ... that of pure water. Alth ... initial toxic increase of ... protein simply diffuses from the blood capillaries), it must be assumed that reflexes also play a certain role in the development of pulmonary oedema. *A proof, among others, of this assumption is* an animal experiment of Jores (1906) who succeeded in producing pulmonary oedema by the irritation of the mucosa of a small bronchial branch.

pulmonary oedema.

These results have recently been confirmed by Smith and Moran (1954): they produced pulmonary oedema, in other cases pneumonia, by the intratracheal administration of sugar.

His experimental investigations into the problem of pulmonary oedema have led *Halmágyi (1954) to the following conclusions:*

1. The appearance of oedema after the intravenous administration of physiological saline depends on the speed and amount of the infu-

2. The infusion of physiological saline ... *of the vagus* leads to pulmonary oedema *without any disturbance or pressure*. This shows that a change in the permeability of the vessel walls must play a certain role in such cases.

3. Disturbance of permeability manifests itself in the protein content of the oedema fluid: different kinds of pulmonary oedema contain equal amounts of protein.

4. There are pulmonary oedemas which arise in spite of perfectly normal pressure in the left atrium (pulmonary oedema produced by α-naphthyl thiourea [ANTU]).

The lung is known to possess an extraordinarily profuse network of lymphatics which, according to Miller (1947), is richer than that of [illegible] Földi, Kepes, [illegible] mption that [illegible] *in the fluid circulation of the lung both under normal and pathological conditions.*

The object of our investigations was to find out whether our theory that [illegible] *latio* [illegible] ments we wish to consider those reported data relating to pulmonary oedema which also take lymph circulation into account. The voluminous literature referring to pulmonary oedema contains very few such works.

Paine, Butcher, Howard and Smith (1949a) substituted Locke's solution, in which an adequate amount of erythrocytes was suspended, for half the blood used for the perfusion of the heart-lung preparation [illegible] pressure was followed by increased lymph flow from the right lymphatic trunk in 1 to 10 minutes.

Blood level in the venous reservoir fell rapidly, indicative of the fact that the amount of blood circulating in the preparation had diminished. The heart did not dilate and pressure remained unchanged: it was in the lung that the amount of fluid increased. During the perfusion of the preparation with diluted blood and while fluid was escaping from the blood into the pulmonary tissue, a slow rise of the protein level in the blood was occurring. Lymph flow remained unchanged nevertheless, and the pulmonary oedema did not decrease. According to Paine and his co-workers, lymph flow remained high in spite of the restored normal level of the plasma protein because the lymphatics had removed the fluid which had entered the pulmonary tissues during the above-described process.

In other experiments Paine and his associates produced congestion in the lesser circulation by increasing the aortic pressure from 90—110 mmHg to 160—180 mmHg, or by increasing venous inflow from 200—300 ml/min. to 600—700 ml/min. As a result of these procedures, the lung became dilated, and crepitation could be heard by direct auscultation. Also in these experiments the fluid level decreased rapidly in the venous reservoir, while (3 to 12 minutes after the appearance of the pulmonary congestion) lymph flow in the right lymphatic trunk became considerably more copious. Lymph was quite clear

at first; it later became *pink and finally dark red*. The lymph contained about 600 000 red blood corpuscles per mm$^3$ in these cases. Warren and Drinker (1942) also observed that a compression of the pulmonary veins led to increased lymph flow in the right lymphatic trunk. Lymph soon became blood stained. *Warren and Drinker* attribute this phenomenon to the hypoxaemia produced by venous congestion and to the consequent increase in capillary permeability.

Paine and his collaborators lay stress on the difference between hypoproteinaemia and venous congestion. If a hypoproteinaemic lung becomes oedematous, it is pale and soft, lymph flow becomes first sluggish and then gradually quicker. Congested lungs are red and swollen, and lymph flow increases suddenly.

Paine and his associates draw the correct conclusion from their observations that *Starling's laws concerning the movement of fluid apply also to the lesser circulation*. Another of their conclusions is that there exists a correlation between the origin and development of pulmonary oedemas on the one hand and the increase of lymph flow on the other: "The existence of a pulmonary oedema was demonstrable whenever lymph flow had become considerably more copious...; increase of lymph flow is a reliable indicator of pulmonary oedema."

The interpretation given by Paine and his co-workers to the connection between pulmonary oedema and the increase of lymph flow is surely wrong. It is clear from their words that they think of two coordinated phenomena, though it is evident that the rise of pulmonary oedema in both types of their experiments is due, *in the last analysis*, to *insufficiency of pulmonary lymph circulation*, presumably due to dynamic insufficiency. If filtration into the pulmonary tissue is increased as a result of higher capillary or lower colloid-osmotic pressure, the lymphatics will have to transport more fluid, and pulmonary oedema appears — as will be seen later — *only when the lymphatic apparatus is no longer able to keep the pulmonary tissue "dry"*.

Cameron and Sheikh mention in their work on pulmonary oedema produced by ammonium-ion that the perivascular and peribronchial lymphatics are already much dilated in an early stage of intoxication, [illegible] the appearance of oedema fluid in the alveoli. This notwith- [illegible] the role of the lymph- [illegible] oedema, nor do they [illegible] f lymph circulation as a factor involved in the origin of pulmonary oedemas.

Cameron and Sheikh distinguish the following types of pulmonary oedema:

1. hydrostatic pulmonary oedema,
2. osmotic pulmonary oedema,
3. neurogenous pulmonary oedema,
4. endotheliogenous pulmonary oedema.

Lorber (1910), studying heart-lung preparations, investigated the role of the lymph vascular system in the production of pulmonary [illegible] at the time of the formation of the pulmonary oedema, he came to the following conclusion: "It is unlikely that the alveolar tissue is provided with a lymph-draining mechanism suitable for the removal of a considerable amount of fluid". This experiments of Lorber has the defect that pulmonary lymph — except in the region of the left apex of the lung — is not removed by the thoracic duct but through the right lymphatic trunk.

Just the opposite conclusion was reached by Richter (1952) who investigated the mechanism of pulmonary oedema caused by α-naphthyl thiourea (ANTU): "Extracellular fluid escapes from the blood capillaries so quickly that the right lymphatic trunk is not able to carry it off. Therefore, part of the fluid enters the alveoli, and part of it fills up the pleural lymphatics". We agree completely with this statement of Richter on the evidence of our own experiments, to be discussed later; we must, however, remark that Richter was not yet in a position to support his claim by direct experiments. Pulmonary oedema produced by ANTU is explained by Drinker and Hardenbergh (1947) in the same sense.

"The lymphatic system of the lung is not capable of conveying large amounts of fluid" — writes Drinker. This is the reason why — following the administration of ANTU — much of the oedema fluid flows off towards the trachea. Drinker's claim that in cases of pulmonary oedema produced by ANTU both the oedema fluid drawn from the trachea and that collected from the pleural transudate show the same protein content, is of pathogenetic significance.

Worthy of note also is Latta's observation (1947): in cases of ANTU intoxication, the first to expand are the perihilar lymph vessels; after this, the alveolar walls become oedematous, and it is only then that pulmonary oedema arises.

It should be noted that pulmonary oedema due to intoxication with ANTU is generally accompanied by hydrothorax. It is possible that this fluid emerges from the superficial lymphatics. Maximov and Bloom (1939) claim that fluid escapes through the lymphatics which run along the pleural veins if the lymph trunks leading to the hilus of the lung are obliterated for some reason. Richter observed the appearance of "fluid lakes" in the surroundings of blood vessels and lymphatics; *these fluid-filled depressions around the hilus are easily visible* through the pleura. "How the fluid escapes from the lymphatics is not known" — writes Richter. The escape of fluid from the lymphatics has been observed by us too (see the physiological part).

According to Courtice (1953), the following factors may lead to pulmonary oedema:

1. Increased permeability of the blood capillaries in the lung.
2. Disturbance of pulmonary lymph circulation.
3. Change of normal pressures in the pulmonary circulation.

1. Cameron and Courtice (1946), as well as Cameron, Courtice and Short (1947), demonstrated that the amount of lymph obtained from the right lymphatic trunk was considerably increased and the lymphatics considerably dilated when they produced pulmonary oedema by phosgene poisoning in animal experiments. If the animals survived the acute stage of poisoning, the protein-rich fluid that had entered the alveoli became slowly absorbed (in about a week). It was shown by Drinker and Hardenbergh (1947) as also by Courtice and Simmonds (1949a) that this protein is absorbed exclusively through the lymphatics, in a native state, i.e. without first being decomposed by proteolytic enzymes. A quick expulsion of the oedema fluid from the alveoli can only be effected by coughing. The removal of protein is a slow process. Courtice's statement that lymph drainage from the lung is completely stopped by anaesthesia or by long immobility in a recum- [illegible] tied on a operation table and remains secumbent during several hours. In this posture, pulmonary oedema develops in the deeper parts of the lung. This is so because a hypostasis forms in these parts with a consequent increase of capillary pressure. Therefore, filtration of fluid increases, whereas the transport of lymph becomes insufficient as a result of immobility. Movement is known to be one of the most important motors of lymph transport in the entire body; that this applies also to the lymph circulation of the lung was demonstrated by Courtice and Simmonds. They introduced dye (T-1824) bound to protein into the alveoli and then observed the appearance of the dye in unanaesthetized animals on walking, during movements and at rest (bound fast). This had already been demonstrated by Shinghu (1908) in respect of the absorption of corpuscular elements.

Drinker and his collaborators (Warren, Peterson and Drinker 1942; Drinker and Warren 1943; Warren and Drinker 1942; Drinker 1945) investigated the effect of hypoxaemia in connection with the genesis of pulmonary oedema. In one group of experiments they made dogs inhale a gas composed of 90% oxygen and 10% carbon dioxide. In this case, the respiratory volume rose to fivefold the normal value. When they inserted a resistance into the inhalation tube by means of a piece of cotton-wool — expiration remaining unhindered — the negative pressure in the thorax rose from 1·6 mm Hg to 9·4 mm Hg. The animals were kept in this condition by means of artificial respiration through a pump for 4 hours and then sacrificed. Neither pulmonary oedema nor pleural transudation could be observed. Thus, forced breathing against resistance without hypoxia did not cause pulmonary oedema, but the volume of lymph flow in the right lymphatic trunk

was found to have increased. Capillary filtration and so also lymph flow were, according to Drinker, increased as a consequence of the changes in pressure, and this was not of a membranogenous character. In another series of experiments the animals inhaled a mixture composed of 10% oxygen and 90% nitrogen; inspiration became difficult also in this case. Of course, hypoxia arose: the average oxygen saturation of the arterial blood dropped to 11.33 volume %. The animals were killed after two and a half hours; the autopsy revealed pulmonary oedema and there was fluid in the pleural cavity *also*; the latter contained, on an average, 5.4 g% of protein and also red blood corpuscles. The lymph flow in the lung was likewise increased, and histological examination revealed dilated lymphatics in the pulmonary parenchyma. Drinker and his co-workers draw the conclusion that hypoxaemia, together with increased intrathoracic negative pressure, augment capillary filtration in the lung to an extent which exceeds the capacity of the lymphatics so that pulmonary oedema results. Drinker declares that increased capillary filtration causes fluid to accumulate in the alveolar septa, part of which passes into the lumen of the alveoli. Consequently, hypoxia arises, or hypoxia already existing is aggravated. Hypoxia leads to increased capillary permeability which, again, leads to a still further increase of capillary filtration.

We must remark that Drinker did not investigate haemodynamic conditions, so that it is possible that increased filtration in his experiments was not, or not *exclusively*, due to an increase in capillary permeability, but may have also been caused by a direct augmentation of filtration pressure.

Courtice and Korner (1952) failed to observe pulmonary oedema when they made animals breath over 5 hours a mixture containing 11% oxygen. Hypoxia accelerated, however, the appearance of pulmonary oedema induced by the infusion of abundant saline solutions; half the amount of fluid sufficed to provoke oedema. According to Courtice and Korner, this effect of hypoxia may also be explained by haemodynamic factors (decrease of cardiac output, vasoconstriction in the systemic circulation, generalized phlebohypertension, congestion in the pulmonary circulation). Appreciation of the results of Drinker and Courtice is rather difficult because these authors combined the inhalation of oxygen-poor gas mixture with the infusion of fluid and not with a forced breathing.

The experiment of Drinker, Warren and Mac Lanahan (1938) is nowadays only of historical interest. These authors ligated the right lymphatic trunk of dogs, tied a cannula into the thoracic duct, and then injected various protein solutions — horse serum, crystalline haemoglobin and crystalline egg albumin — into the alveoli through the trachea. Later they examined the appearance of these proteins in

lymph. "In other areas of the organism, one of the most important tasks of the lymphatic system consists in removing proteins from the interstitial spaces" — so they wrote — "while conditions for the formation [illegible] compelled : [illegible] vessels, as [illegible] of inflammation, haemorrhage or when foreign substances are deposited". Drinker's statement that conditions in the lung are not favourable for the formation of tissue fluid is based on observations according to which capillary pressure in the lung is very low: 5—10 mm Hg, i. e. considerably lower than the colloid-osmotic pressure (Hellems, Haynes, Dexter and Kinney 1948; Dexter, Dow, Haynes, Whittenberger, Ferris, Godah and Hellems 1950). For a substance to pass into the lymphatics of the lung from the alveoli it is necessary to penetrate *through certain pulmonary tissues, as the lymph capillaries do not* extend beyond the region of the alveolar ductules. Small inorganic particles and bacteria seem to take this route into the breathing lung, but the fluid of the alveoli is not removed by the lymphatics. Not even strong respiratory movements which usually facilitate the entrance of intra-alveolar particles into the lymphatics of the lung suffice to propel the fluid into the pulmonary lymph channels.

Drinker and his associates seem to have forgotten that the *major part of the pulmonary lymph is drained by the right lymphatic trunk*; they ligated this vessel and examined the lymph of the thoracic duct. What they actually investigated, therefore, after the obstruction of the pulmonary lymph circulation, was the absorption of proteins from the alveoli into the blood and also into the lymph source of the thoracic duct, i.e. especially towards the abdominal organs.

Thus, the experiments of Drinker and his co-workers tell us nothing about the function of the pulmonary lymphatics under normal conditions. In 1942, Drinker himself recognized the fundamental mistake of these experiments.

It has long been known that the absorption of fluids from the alveoli is a fairly simple process. The first pertinent data in this respect are those of Claude Bernard from the year 1857. It was stated by Colin in 1873 that 12 litres of water could be intratracheally administered to horses during 3 1/2 hours without any harmful effect. Laqueur (1919) gave a rabbit (with a body weight of 1·5 kg) 20 ml of water intratracheally; after the lapse of 30 minutes, all traces of dyspnoea had already disappeared.

We must also deal with the question of intrathoracic negative pressure in cases of *existing* pulmonary oedema. It has already been mentioned that in cases of experimental pulmonary oedema escape of fluid from the subpleural lymphatics into the pleural cavity was observed. The phenomenon that transudation into the pleural cavity follows the appearance of dyspnoea in patients suffering from pulmonary oedema is attributed by Graham (1921) to the effect of intrathoracic nega-

tive pressure on pulmonary fluid circulation: negative intrathoracic pressure, steadily increasing because of the dyspnoea, *aspires* the fluid from the pulmonary tissue and the engorged subpleural lymph channels. The fluid so lost is then replaced by filtration.

Consideration has already been given to the fact that lymph circulation in the lung is maintained by respiratory movements. Drinker (1945) declares that lymph circulation in the lung will practically cease if oxygen is supplied by insufflation. Drinker is therefore right to conlcude that the therapeutic effect of pneumothorax is principally due to the accumulation of lymph in the immobilized parts of the lung which are eliminated from the process of breathing: chronic lymph congestion invariably leads to fibrosis and cicatrization (cf. the experiments of Courtice quoted above). Schaklein (1950) also shares the view that "the movement of the pulmonary lymph in a closed [illegible]

*the occlusion of the pulmonary lymphatics in animal experiments.*

Dogs were used in our experiments. Having anaesthetized the animals with aether-oxygen and performed thoracotomy in the right IIIrd intercostal space, we tied off the right lymphatic trunk, the thoracic duct and, in certain cases, also some larger tracheo-bronchial lymph nodes. Also the lymph node to the left of the superior ena cava, directly above the heart, was ligated. After closing the chest, we aspired carefully all the air from the thoracic cavity. We sacrificed 6 animals 24 hours, an animal 10 days and an animal 12 days after the operation. Two animals were killed by air embolism, the others by the i.v. administration of evipan.

Autopsy revealed pronounced congestion in the occluded lymph paths. In order to ascertain whether or not pulmonary oedema had developed we resorted to histological examination as the only exact and reliable procedure.

The recently-adopted body weight/lung weight index is we think wholly unsuit- [illegible]

It can be seen from Table 63 that 3 of the 8 cases belonged to group I and 4 to group II. In one case, no pulmonary oedema had developed. In 4 cases, we performed sham operations: thoracotomy was carried out as in the other experiments, the thorax was kept open and the

artificial respiration maintained approximately as long as in the other operations; the pneumothorax was then stopped and the thoracic cavity closed. No pulmonary oedema arose in these 4 control cases. (Table 63 and Figs. 200—202).

TABLE 63

| | Number of cases | Macroscopic oedema | Microscopic oedema | | | |
|---|---|---|---|---|---|---|
| | | | 0 | I | II | III |
| Ligation of lymphatics | 8 | 2 | 1 | 3 | 4 | 0 |
| Sham operated controls | 4 | 0 | 4 | 0 | 0 | 0 |

Before interpreting these experiments in the sense that [illegible] sion of the lymph circula[illegible] ary oedema, anot[illegible] [illegible], essentially, consisted [illegible] of the left subclavian vein with consecutive congestion in the thoracic duct, and the appearance of chylous pneumonia. The authors assumed that, as a consequence of the congestion in the lymphatic system, the chyle had passed in a retrograde manner [illegible] t into the pulmonary [illegible]

We there[illegible] fat stains. [illegible] [illegible] of the alveoli. It was only in a few larger efferent lymph trunks that fat could be demonstrated (Fig. 203).

Hence we [illegible] of the l[illegible] leads to [illegible] conclusions.

*First, the lung is, after all, not a "d[illegible]"* [illegible] [illegible] and — as is the case elsewhere in the organism — all the protein and part of the fluid has to be carried off by the lymphatics in the lung also. If the lymphatics are occluded, their function is not taken over vicariously by the blood vessels, but the protein-rich fluid enters the alveoli and pulmonary oedema arises. It should be remarked that the alveoli which are filled with oedema fluid do not, of course, participate in the exchange of gases. If Drinker is right in emphasizing in his monograph on pulmonary oedema (1945) that hypoxaemia increases the permeability of the blood capillaries of the lung, then the protein content of capillary filtrate must augment and so the oedema become aggravated.

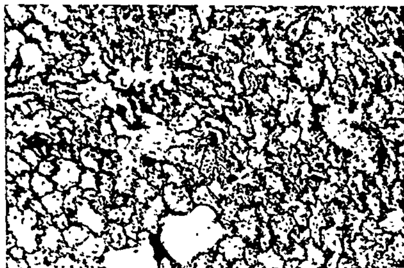


*Fig. 200.* Pulmonary lymph congestion. Groups of oedematous alveoli

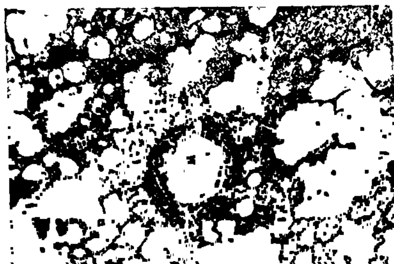


*Fig 201.* Pulmonary lymph congestion Confluent pulmonary oedema

Relying on our experiments and a critical survey of literature we believe that neither the classification of Cameron and Sheikh, nor that of Courtice appears to us satisfactory. Cameron and Sheikh's classification does not take into account the disorder of the pulmonary lymph



*Fig. 202.* Pulmonary lymph congestion. Dilated lymphatic and groups of oedematous alveoli

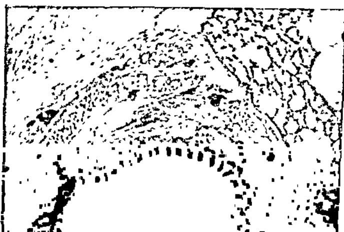


*Fig 203.* Pulmonary lymph congestion. Sudan-positive substance demonstrable in the larger efferent lymphatics

circulation. Though Courtice's classification does not disregard the lymph circu ... 
We sugg ... athogenesis of pulmonary ... *e is a discrepancy in the lung between the production and the removal of capillary fil-*

*trate, i.e. in all cases where the lymphatic system of the lung is insufficient and not capable of keeping the lung "dry".*

*Whether the prime mover is a decrease in colloid-osmotic pressure, an increase in the permeability of blood capillaries in the lesser circulation or the aspiration of water, the appearance of pulmonary oedema is, in the last analysis, always a result of some kind of insufficiency of the pulmonary lymph circulation.*

However, in order to furnish conclusive proof it seemed necessary to prove our thesis from still another angle (Földi, Kepes, Robicsek, Rusznyák and Szabó 1955). If it is true that it really depends on the sufficiency of the lung's lymphatic apparatus whether or not a change in the pressures prevailing in the pulmonary circulation will induce oedema, the obliteration of the pulmonary lymphatics in a congestion of the lesser circulation must surely lead to a much graver pulmonary oedema whenever the mechanical insufficiency of the lymphatic system is brought about with Starling's equilibrium remaining normal.

We employed two methods for the overthrow of Starling's equilibrium in the pulmonary circulation: we produced artificial cardiac failure in dogs or performed bilateral cervical vagotomy on the animals.

a) We provoked cardiac failure in 8 cases: mitral insufficiency in 6 and mitral stenosis in 2 dogs.

Mitral insufficiency was induced in the following manner: we opened the thorax in the IVth intercostal space and exposed the heart. After inserting two supporting threads into the anterior wall of the left ventricle, we penetrated with a pair of fine scissors between the two threads into the left atrioventricular orifice and injured the mitral valve. The degree of insufficiency so produced was indicated by a water manometer tied into the left atrium.

Mitral stenosis was provoked by pulling the left auricle into the mitral orifice. It was then fixed in this position by a few stitches so that the mitral orifice became plugged and so considerably narrowed.

A few weeks, in some cases a few months, after the production of the cardiac [illegible] compensating function by increased fluid transport.

(It should be noted that, at commissurotomy on patients with mitral stenosis, the pulmonary lymphatics were frequently seen to be engorged with lymph to the point of bursting.)

We then ligated the right lymphatic trunk, the thoracic duct and the lymph nodes in the anterior mediastinum, aspired the pneumothorax and closed the thoracic cavity.

The animals were sacrificed 24 hours later with intravenous evipan injection; we fixed their lung in hot formalin and analysed it histologically.

Histological examination showed that prior to the ligation of the lymphatics no pulmonary oedema had developed in the dogs suffering from valvular lesion; after the ligation of the lymphatics the gravest

TABLE 64

*Effect of ligature of lymphatics*

| No | Pulmonary lymph congestion | Degree of oedema | Other changes in the lung |
|---|---|---|---|
| 1 | ++ | III | oedematous "intraadventitial spaces" |
| 2 | ∅ | I | — |
| 3 | few, only peribronchially visible lymphatics | ∅ | — |
| 4 | — | I | haemorrhage, congestion |
| 5 | ∅ | I | — |
| 6 | + | III | congestion, haemorrhages |
| 7 | + | III | congestion |
| 8 | + | III | congestion |

pulmonary oedema of type III was encountered in 4 of 8 cases, a mild oedema of type I in 3 cases, and no oedema in one case (Table 64).

b) We tied off the pulmonary lymph vessels of vagotomized animals in another group of experiments. As is known, a bilateral division of the vagus does not cause pulmonary oedema in the dog. It was, however, demonstrated by Halmágyi (1954) that vagotomy makes the animals susceptible to oedema, probably on account of increased capillary permeability in the lesser circulation.

After having tied up the lymph channels of the lung in the described manner and having closed the thorax, we isolated and ligated both vagi in the neck. The animals were killed by i.v. evipan injection 24 hours later. We performed altogether 7 experiments of this kind.

Histological examination gave the following results: the gravest type of pulmonary oedema, which in some instances extended even to the bronchioles, was encountered in 3 out of 7 cases. Oedema belonging to group II was found in one case. No pulmonary oedema was observed in 2 cases, while — in one case — the oedema could not be classified because of confluent bronchopneumonia, a well-known complication of vagotomy (Table 65).

We had to elucidate yet another question (Földi, Kepes, Robicsek, Rusznyák and Szabó 1955). The doubt may arise that pulmonary capillary pressure was *increased* in our experiments, so that the strain to which the circulation was exposed by the grave surgical intervention

TABLE 65

*Division of vagus and ligature of the lymphatics*

| Nº | Pulmonary lymph congestion | Degree of oedema | Other changes in the lung |
|---|---|---|---|
| 1 | + | III | oedematous "intraadventitial spaces" |
| 2 | ø | III | — |
| 3 | ø | inflammatory | confluent bronchopneumonia |
| 4 | ø | ø | focal bronchopneumonia |
| 5 | ø | ø | venous congestion |
| 6 | + | II | — |
| 7 | + | III | oedematous "intraadventitial spaces" |

provoked congestion in the lesser circulation or perhaps it is possible that the lymph congestion itself produced increased capillary pressure.

Therefore, we induced mitral insufficiency in four more dogs. Using a heart catheter, we ascertained the pulmonary capillary pressure by an electromanometer 10 to 14 days after the intervention. After this, we produced lymph congestion in the lung and examined the pulmonary capillary pressure 24 hours later once more.

*Pulmonary capillary pressure was found to be unchanged in every case which shows that it is really lymph congestion which must be regarded*

TABLE 66

| No. | Ligation of the lymphatics | Capillary pressure in mm Hg | |
|---|---|---|---|
| | | systolic | diastolic |
| 1 | before | 15 | 10 |
| | after | 15 | 8 |
| 2 | before | 10 | 4 |
| | after | 8 | 0 |
| 3 | before | 21 | 10 |
| | after | 10 | 0 |
| 4 | before | 11 | 8 |
| | after | 10 | 8 |

TABLE 64

*Effect of ligature of lymphatics*

| No | Pulmonary lymph congestion | Degree of oedema | Other changes in the lung |
|---|---|---|---|
| 1 | ++ | III | oedematous "intraadventitial spaces" |
| 2 | ø | I | — |
| 3 | few, only peribronchially visible lymphatics | ø | — |
| 4 | — | I | haemorrhage, congestion |
| 5 | ø | I | — |
| 6 | + | III | congestion, haemorrhages |
| 7 | + | III | congestion |
| 8 | + | III | congestion |

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| 2 | ∅ | III | — |
| 3 | ∅ | inflammatory | confluent bronchopneumonia |
| 4 | ∅ | ∅ | focal bronchopneumonia |
| 5 | ∅ | ∅ | venous congestion |
| 6 | + | II | — |
| 7 | + | III | oedematous "intraadventitial spaces" |

provoked congestion in the lesser circulation or perhaps it is possible that the lymph congestion itself produced increased capillary pressure.

Therefore, we induced mitral insufficiency in four more dogs. Using a heart catheter, we ascertained the pulmonary capillary pressure by an electromanometer 10 to 14 days after the intervention. After this, we produced lymph congestion in the lung and examined the pulmonary capillary pressure 24 hours later once more.

*Pulmonary capillary pressure was found to be unchanged in every case which shows that it is really lymph congestion which must be regarded*

TABLE 66

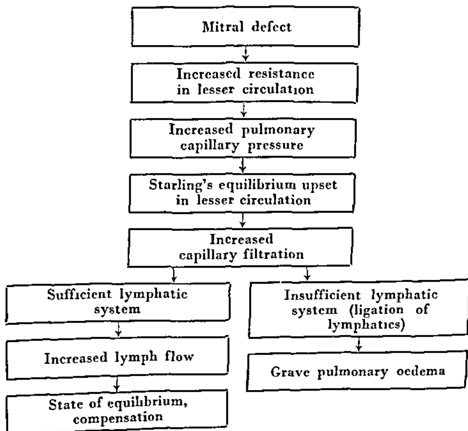
| No | Ligation of the lymphatics | Capillary pressure in mm Hg | |
|---|---|---|---|
| | | systolic | diastolic |
| 1 | before | 15 | 10 |
| | after | 15 | 8 |
| 2 | before | 10 | 4 |
| | after | 8 | 0 |
| 3 | before | 21 | 10 |
| | after | 10 | 0 |
| 4 | before | 11 | 8 |
| | after | 10 | 8 |

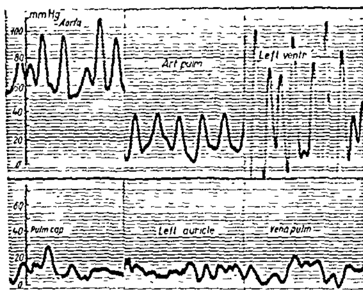
*as responsible for the production of pulmonary oedema and not a change in the haemodynamics of the lesser circulation* (Table 66; Figs. 204, 205)

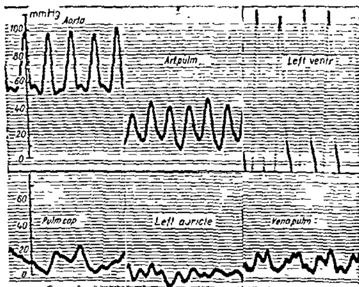
These experiments were founded on the assumption that in the extraordinary conditions in which the lymphatic apparatus of the lung has to perform a particularly difficult task, a ligation of the pulmonary lymphatics ought to provoke a graver pulmonary oedema than that obtained in those experiments in which the lymphatics of intact animals are obstructed, always supposing that we are right in thinking that, in the given circumstances, the lymph vascular system of the lung protects the organism from the development of pulmonary oedema.

We succeeded in substantiating our assumptions by way of experiments. Whereas a "simple lymphatic obstruction" in normal dogs did not produce pulmonary oedema of type III, the "combined intervention" resulted in pulmonary oedema of this type both in dogs with valvular lesion and in vagotomized dogs.

Our experimental results justify the following diagrammatic summary of the origin of pulmonary oedema in our cases of mitral defect:

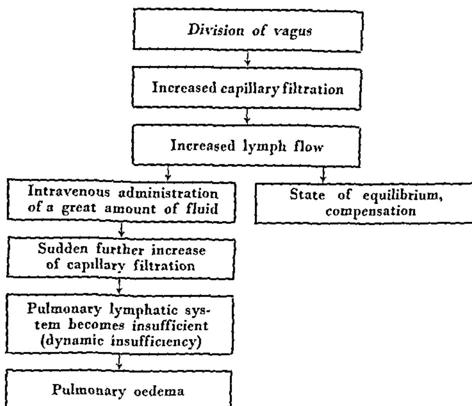





*Figs 204.* and *205* Haemodynamic conditions before and after ligature of pulmonary lymphatics in dogs with bicuspid insufficiency

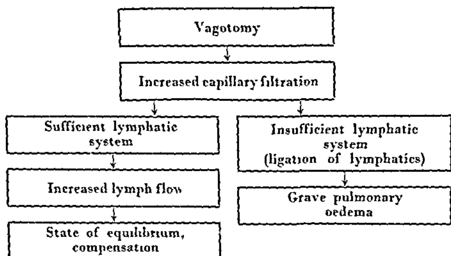
In the experiments of Halmágyi, carried out with vagotomy and the infusion of fluid, pulmonary oedema was produced by means of the following mechanism:




In connection with our experiments, the question arises: why did not "combined intervention" always cause pulmonary oedema? Does it perhaps mean that our theory is wrong after all and that the lymphatic apparatus of the lung is not so very important for pulmonary *fluid circulation under normal and pathological conditions?*

[illegible] has never [illegible] und that [illegible] oedema, no lymph congestion was *demonstrable either. However,* this was simply due to the fact that — having regard to the extraordinary variability of the anatomy of lymphatic system — the ligation of the right lymphatic trunk, of the thoracic duct and of anterior mediastinal lymph nodes which had been *found* was not sufficient to produce lymph congestion in the lung. In view of the variability of the anatomy of the

lymphatic system, the frequently wide extension of anastomoses, it is not surprising that the "simple ligation of lymphatics" (in this group also we observed one case out of eight in which no pulmonary oedema appeared) or the "combined intervention" did not always produce milder or graver pulmonary oedema; it is rather the reverse: it is quite surprising that a ligation of the lymphatics actually produced such a congestion of lymph as to induce pulmonary oedema. [illegible] remember that Drinker and his [illegible] form [illegible] tion [illegible] pathologic progress can [illegible] the following manner:




As already mentioned, we found the pulmonary lymphatics conspicuously wide not only in dogs with cardiac failure but also in human subjects suffering from mitral stenosis. The question may be raised why both anatomists and surgeons have, so far, failed to describe this phenomenon, although mitral defects and congestions in the pulmonary circulation account for a very considerable percentage of deaths, while—on the other hand—hundreds of commissurotomies are nowadays performed in many clinics.

The answer to this question is [illegible] lymphat[illegible] hand, t[illegible] altogether, and on the other [illegible] become empty and collapse very rapidly after death. We have observed this phenomenon more than once:

immediately after death we saw markedly dilated lymphatics, but by the time we were able to get our apparatus ready to photograph this fascinating picture the lymphatics were already empty. We believe that the main factor responsible for this phenomenon is a postmortal

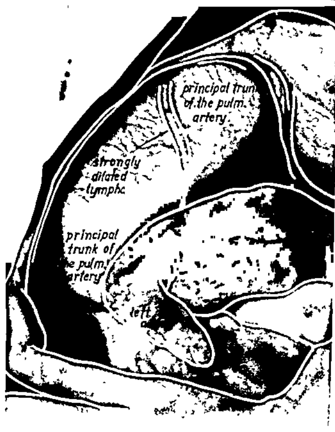



*Fig. 206.* Dilated pulmonary lymphatic in mitral stenosis
Photographed during operation

increase in the permeability of the lymphatics (cf. our work referred to in the physiological chapter): the lymph simply extravasates from the lymphatics into the surrounding tissues. Besides, it is known that the flow of lymph towards the venous system continues for some hours even after death. Nor do surgeons, as a rule, give much heed to the pulmonary lymphatics during operation.

The question arises: which kind of insufficiency of the pulmonary lymph circulation may play a role in human pathology? We have to

rely on mere hypotheses in this respect. We believe that dynamic insufficiency should be considered in the first place: it is by way of *diffusion* that fluids must gain access to the region of the alveolar ducts whence the lymph vessels issue. Moreover, the right lymphatic trunk is an especially narrow vessel. However, *lymphangiospasm* — a mechanical-functional insufficiency — is also conceivable: we know that stimulation of the sympathetic nervous system, capable of producing neurohaemodynamic pulmonary oedema, provokes also lymphangiospasm; the right lymphatic trunk possesses a comparatively well-developed musculature. Sarnoff, Goodale and Sarnoff (1952) obtained satisfactory results from the application of ganglion-blocking drugs in cases of human pulmonary oedema. It is possible that both this therapeutic effec
— at least partly
to the results of
experimental demonstration of this hypothesis is unfortunately very difficult because all these drugs produce, or may produce, a haemodynamic effect as well.

Relying on a critical survey of the literature and our own investigations, we suggest that pulmonary oedema may arise if

1. *filtration from the pulmonary capillaries increases*
   a) *by augmented capillary permeability,*
   b) *by increased capillary pressure,*
   c) *by decreased colloid-osmotic pressure ;*

2. *fluid containing protein or fluid which becomes rich in protein secondarily penetrates into the alveoli from the outside,*
*provided the pulmonary lymphatic system is insufficient in the above sense in all these cases ;*

3. finally, *if a mechanical insufficiency of the pulmonary lymphatic system is present.*

As to point 2, we must emphasize that if it is pure water, i.e. a fluid free of protein, which passes into the alveoli, it need not, in itself, give rise to pulmonary oedema, since — except if the fluid suddenly inundates most of the alveoli which, of course, must lead to death by asphyxia — water is absorbed by the blood capillaries (see Colin's above-quoted data). Pulmonary oedema arises when the amount of fluid is
there is sufficient t
capillaries. This occ
according to Drinker, still more in cases of anoxia. If this happens, there is only the lymphatic system, a slowly absorbing apparatus, which can carry off the protein-rich fluid. It is for this reason — as Cour
prote
and

protein-free fluid taken up by the blood capillaries; what is slowly absorbed is the fluid which already contains protein and is taken up by the lymph vessels. Of course, beside this mechanism a certain role must be attributed also to the above-mentioned reflexes which proceed from the wall of the water-filled bronchioles, and we believe we are justified in assuming that lymphangiospasm, provoked by the strong irritation of the sympathetic nervous system, is also involved.

It is worth while briefly discussing that form of pulmonary oedema, which arises in patients suffering from mitral stenosis as a consequence of psychic excitement. Scherf and Boyd (1948) explain this paroxysmal pulmonary oedema by tachycardia, with the inflow of great amounts of blood into the lesser circulation; this augments intrapulmonary congestion, capillary pressure, to such an extent as to provoke pulmonary oedema. Matters are, however, not quite so simple as is proved by the fact that the administration of morphine has been found to give satisfactory results in the therapy of paroxysmal pulmonary oedema. This means that this form of pulmonary oedema is of a neurohaemodynamic character and that the dynamic insufficiency of the lymphatic apparatus (or, possibly, its mechanical insufficiency: irritation of the sympathetic → lymphangiospasm) is naturally involved in its production. If mitral stenosis, i. e. increased pressure in the lesser circulation lasts long, the attacks of pulmonary oedema become less frequent, to cease later altogether. Scherf and Boyd suggest that a sclerosis of the pulmonary blood vessels is developing and leading to decreased permeability in these cases.

In conclusion, we wish to discuss another problem, viz. the question of pulmonary oedema arising in Starling's heart-lung preparation. As has already been mentioned, pulmonary oedema appears sooner or later in every heart-lung preparation. This lung oedema is generally attributed to the action of toxic substances present in the blood used for perfusion. We think that there is also another factor involved in the development of pulmonary oedema in heart-lung preparations, namely the circumstance that the *Vv. cavae are ligated* in such preparations so that the drainage of the large lymph trunks into the veins becomes obstructed. Added thereto is the effect of artificial respiration; we know from Drinker's experiments that it leads to increased lymph [illegible]

lung becomes oedematous in heart-lung preparations but also the water content of the cardiac musculature increases. We have already seen in the chapter on the lymph circulation of the heart that lymph congestion leads to myocardial oedema. Hence, in the sense of this concept, both the pulmonary oedema appearing in Starling's preparation and the oedema of the cardiac musculature are due to the same cause. It is, of course, still possible that the toxic effect of the blood used for perfusion is also involved.

## LYMPHATIC SYSTEM OF THE LUNG IN PNEUMONIA

The various forms of the insufficiency of lymph circulation have been extensively discussed in the general part of this work. All we would add here is that intralymphvascular coagulation and the subsequent organization of the inflammatory products that have passed into the lymphatics must lead to the gravest form of mechanical insufficiency of lymph flow. The possibility of its appearance in the lung is proved by the observation that in lobar pneumonia, in the pathology of which the pulmonary lymphatic system is invariably involved, fibrinous exudate can sometimes be demonstrated not only in the alveoli but in the lymphatics as well; histological analysis shows that the endothelial cells of the pulmonary lymph vessels become dilated, even desquamated. The network of subpleural lymphatics is frequently well-visible. Therefore, both *lymphangitis and perilymphangitis are really present in pneumonia*. It was observed long ago (see Kaufmann 1911) that the regional lymph nodes of the lung become strongly tumescent at the stage of "engoument", a phenomenon ascribed to increased absorption by the lymphatic apparatus of the lung. The swelling of the lymph nodes subsides in the phase of hepatization and reappears once more in that of resolution. Staehelin (1930) claims that proliferative inflammatory processes inside the lymphatics and perilymphangitic phenomena may lead to obliteration of the pulmonary lymphatics.

Also noteworthy in this connection is Stein's observation (1948): anatomical lesions of the pulmonary lymphatics can be demonstrated in children afflicted with bronchopneumonia. According to Baum (1926), the pulmonary lymphatic system always participates in the bronchopneumonia of horses and pigs. It is evident that pneumonia cannot heal unless the lymphatic apparatus is working with full transport capacity; it is known that only an insignificant part of the exudate can be discharged by expectoration.

*Carnification* in pneumonia is generally interpreted by the assumption that, for some reason, liquefaction of the fibrinous exudate by the leukocytes fails to occur, so that the exudate arrested in the alveoli begins to be organized. We have encountered no histological proof of this theory in the available literature, *nor do we think that it can be proved at all.* If a patient dies of pneumonia and if the histopathological examination reveals the alveoli to be filled with a precipitated fibrinous mass, it may mean that the liquefaction of the exudate had not yet taken place, but there is no evidence to show that this would not have happened if the patient had survived. If, on the other hand, it is found at autopsy that a carnification of the pneumonia had already occurred and that connective tissue had developed in the alveoli at the site of the exudate, it is not admissible to conclude backward as to whether the exudate had become liquefied or not.

This question could only be elucidated if repeated biopsies were performed in the course of the pneumonia which is evidently impossible.

We consider it very probable that a mechanical insufficiency of the lung's lymphatic apparatus may play an important part in carnification: if the exudate filling up the efferent pulmonary lymph trunks becomes first coagulated and then organized, the result must undoubtedly be first carnification and then a cirrhosis of the lung.

A statement contained in Kaufmann's text-book is very interesting in this connection: in the *emphysematous* lung — in which, simultaneously with the general disintegration of the pulmonary tissue, the pulmonary lymphatics become destroyed — the exudate "gets stuck" in cases of pneumonia; it undergoes carnification. Noteworthy is also a statement of Buday (1929): *"Chronic transudative pneumonia develops if the absorption of the transudate is delayed for some reason, e.g. because of an occlusion or a cicatricial compression of the pulmonary lymphatics. It is in such cases that we can observe the pathological picture which used to be termed Pneumonia gelatinosa"*.

Recently, Yessipova (1952) concerned herself with the pathogenesis of the pulmonary cirrhosis and pneumosclerosis appearing in connection with cases of unspecified chronic pneumonia. Yessipova claims that in unspecific chronic pneumonia pneumosclerosis may be produced as a result of different pathological processes. These are the following:

1. organization or carnification of the exudate;
2. interstitial pneumonia or productive inflammation of the interalveolar septa;
3. cicatrization of atelectatic regions of various origins;
4. *thickening and coarsening of the interstitial layers of the lung in which the major lymphatics run;*
5. thickening, collagenization of the alveolar septa.

Of interest is Yessipova's statement in connection with bronchiectatic pericavernous pulmonary sclerosis:

"If the cavern is closed, perilobular bundles can only be observed directly beside the cavern. If, however, the cavern is open and if the bronchi are inflamed, the perivascular bundles grow coarser far beyond the borders of the cavern, in accordance with the alteration of the bronchi. This is explainable by the fact that sclerosis develops along the lymphatics which are running in the peribronchial, perivascular and perilobular tissue, forming a uniform system."

Pulmonary sclerosis may, according to Yessipova, also be produced on the ground of acellular fibrosis. Numerous authors acknowledge the existence of acellular fibrosis. Yessipova attributes the perilymphvascular appearance of sclerotic phenomena to the fact that "the protein required f[illegible] the construction of the ground substance of the con-

nective tissue originates from the lymph which flows in great amounts from the region of the centre of inflammation. This lymph is known to contain much more protein than under normal conditions. In the region of the inflammatory centre, lymph clefts and lymph vessels are easily visible: they stain with eosin intensively and are, therefore, filled with protein-rich fluid. It is just along the clefts and vessels that the transformation and accumulation of the fibres of connective tissue begin... That interstitial substance develops from the proteins of the lymph is shown by the pictures in which an amorphous mass, staining weakly with eosin, is seen to be deposited under the endothelium of the lymph vessels. *The protein-rich fluid seems to diffuse through the thin wall of the lymphatics* (!). Sclerosis is most intensive in the neighbourhood of the regions which are in purulent inflammation; lymph coming from such regions shows the highest protein content".

Sclerotic phenomena develop both proximally from the focus of inflammation, towards the hili and, distally, in the direction of the pleura. Yessipova explains the phenomenon — with reference to Iwanow and Ravitch-Shtcherbo — by retrograde lymph flow. She gives a similar interpretation to the development of sclerosis in the pleura which is rich in lymphatics.

Yessipova declares that, in purulent bronchitis, atelectatic regions in the lung develop rapidly. Sclerosis of that portion of the pleura which belongs to the inflammatory segment leads to the same result: "As a consequence of the fixation of pulmonary tissue, also lymph and [illegible]
still [illegible]
bloo[illegible]
mark[illegible]
the conditions of intensive pulmonary sclerosis are created."

This is why in the lung — which is transformed into a morass by the lymph more than any other organ (Shtefko) — a suppuration and fixation of the pulmonary tissue give rise to the appearance of such coarse and massive cicatricial areas as cannot be encountered anywhere else in the organism".

## ROLE OF THE PULMONARY LYMPHATIC SYSTEM IN PNEUMOCONIOSIS

Insoluble inorganic substances, e.g. dust, are removed from the alveoli by the lymphatics. Tendeloo (1902) claims that, while part of the dust streams freely in the efferent lymphatics, another part of it [illegible]

dust and that transported intracellularly a part gets into the pulmonary lymph nodes, while another part is carried by the lymph flow into different portions of the lung and even into the pulmonary and the

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Lime dust and, to a still larger extent, quartz dust provoke inflammation, and cicatrization even more frequently.

Pozharski, Toltchskaya and Shilova suggest that the absorption of silicon dioxide dust along the lymph vessels leads gradually to lymphangitis and perilymphangitis which, in their turn, give rise to the sclerosis of the perivascular and peribronchial connective tissue. While silicon dioxide is being propelled by the lymph stream, it settles in the lymph nodes of the hilus and the subpleural lymph pools. Lung tissues are damaged, congestion arises, since the absorption of metabolites in the lymphatic system is limited on account of the fact that, in silicosis, lymph vessels become diseased very soon.

Dvizhkov (1951) fully discussed the alterations of the lymph vascular system as encountered in cases of silicosis. He refers, by way of introduction, to the experiments of Peissachowitsch who made dogs inhale air contaminated by the dust of a porcelain factory, and found hyper- [illegible]

[illegible]

Dvizhkov demonstrated that, in silicosis, pathological lesions were observable also in the lymphatics of the *upper respiratory tracts* : "In the submucosa of the trachea and the larger bronchi, dilated lymph vessels can be seen whose lumina contain ovally extended large cells. Their nucleus is round and oval, their protoplasm contains numerous, dull-yellow dust grains. On the other hand, proliferation and desquamation towards the lumen of the lymph vessels can be seen in the lymphatic endothelium which is overloaded with dust containing silica, and also phagocytes with engulfed dust coming from the lung [illegible] mphatics

[illegible] intrapulmonary lymphatics is perceptible in the initial phase of pneumoconiosis; the lumen of the dilated lymphatics is filled with ovally-extended cells which — containing dust and having polymorphous nuclei — usually adhere closely to the capillary wall. "Their endothelium has grown cubical and round and has invaded the lumen. There are two sorts of cells here: dust cells of the histiocyte type, and the epithelial cells of the lymphatics."

Hence, it seems that also the endothelial cells of the pulmonary lymphatics become diseased in the initial phase of pneumoconiosis; Dvizhkov's description strikingly recalls the picture we observed in the lymphatics of the thyroid gland in cases of colloid goitre, and also in certain pathological phenomena in adults (cf. the chapter on thyroid).

Dvizhkov remarks that the dilatation of the perivascular pulmonary lymph paths, "with great masses of dust cells, proliferating and desquamated epithelial cells in the lumen, may become so pronounced as to be reminiscent of the destructive perivascular spread of pulmonary cancer".

costal pleura. Part of the dust gets trapped in the pulmonary tissue, provoking there reactive inflammation. With the disintegration of the cells, the intracellular dust particles become free and they are then phagocytosed again by other cells. Very important is Staehelin's statement (1925) that lymphatics may become obliterated if they are engorged with dust-filled cells or free dust particles.

Dust grains finding access to the bronchial lymph nodes first enter the perifollicular sinus, to fill up later the entire lymph node (Arnold 1890).

Worthy of mention is the observation of Meneely, Qualls and Curtis (1951): they administered radioactive gold sol intratracheally to dogs and found that the vehicle, the water, was quickly absorbed; the radiation of the radioactive gold in the tracheobronchial lymph nodes attained, however, a significant degree only after 10 to 14 days. Radioactive silver is transported more quickly by the lymphatics. Hahn and Carothers (1951), as well as Hahn, Rouser, Bromitt, Moorehead and Carothers (1952), introduced a suspension of radioactive silver colloid into the alveoli of dogs. In this case, intensive radioactivity in the tracheo-bronchial lymph nodes became perceptible after two days.

These authors think that the introduction of slowly-absorbed radioactive gold by means of the bronchoscope may, in cases of pulmonary tumours, prove suitable for the therapeutic irradiation of the lung parenchyma, while radioactive silver may be used for the irradiation of the regional lymph nodes.

Durable inhalation of silicon dioxide leads to a chronic lymphangitis, perilymphangitis and, subsequently, to the obliteration of the vessels which carry this substance (Karpilowski 1939, see Rotenberg 1952; Pozharski, Toltchskaya and Shilova 1950; Dvizhkov 1951). There can be no doubt that the mechanical insufficiency of the pulmonary lymph circulation, brought about in this manner, must be regarded as the principal factor responsible for the cirrhosis of the pneumoconiotic lung. We are in agreement with Staehelin's statement that, as long as only negligible quantities of dust are inhaled, the lymphatic system is able to remove the dust from the alveoli so that the lung parenchyma remains intact. If larger amounts of dust are inspired for a long time, the lymphatic apparatus will become insufficient, dust particles will remain in the pulmonary tissue and induce [illegible] inflamma- [illegible] evidently slight, so that severe anthracosis is frequently encountered in cases where the functioning of the lung does not seem to be impaired.

Dvizhkov also investigated the changes that occur in the lymph nodes in cases of silicosis. What one observes first are the augmentation of the reticulo-endothelial elements of the sinuses, the tumefaction, proliferation and the subsequent desquamation of the cells; dust grains are demonstrable in the protoplasm. It is from this picture of sinal catarrh that, by degrees, cicatrization and later even the necrosis of lymph nodes develops. In the stage of necrosis "collagenous fibres appear in the sinuses first in a small amount; their number then becomes greater until they surround the follicles on all sides. Perifollicular sclerosis may assume very considerable proportions. It is surrounded on all sides by connective tissue which gives rise to a picture of *annular sclerosis*, the capsule becomes thicker, its capacity of contraction diminishes and so the lymph flow is *slowed down*". Changes in the necrotic lymph nodes are — according to Dvizhkov — due partly to the direct effect of the great amount of silica dust and partly to the deficient blood supply in the newly-formed young connective tissues owing to the vascular lesions. Dustcontaining silica induces first sclerosis and then a necrosis of the vessel walls; these allow the dust to pass into the blood stream which carries it even to distant parts of the organism.

Very noteworthy is the following statement of Dvizhkov: "...the thoracic lymph vascular system, i.e. the draining apparatus of the respiratory organs, becomes practically incompetent in the advanced stage of silicosis. The moment when this happens is very important for the subsequent condition of the pulmonary tissue, as concomitant diseases — bronchial catarrh, pneumonia, and tuberculosis — may easily develop We must assume that the elimination of the thoracic lymph vessels means the beginning of the tragic end awaiting patients afflicted with silicosis".

Erdélyi (1953) recently investigated in our Institute the problems of pneumoconiosis, i.e the silicosis of enamel workers. In Erdélyi's work it is stated that silicosis is characterized by the impairment of the pulmonary lymphatics: "A cicatricial coat develops in the surroundings of the lymph vessels, the atrophy of which leads to a congestion of lymph. Since it is in the lymph nodes that slag is arrested in the lung, we encounter — especially in the perihilar lymph nodes — notable alterations which are important from the viewpoint of differential diagnosis. They grow larger, become indurated and give a very hard calcium-like shadow in X-ray pictures. This hard lymph-node shadow is composed of three factors. One is the inhaled foreign particle arrested in the lymph node; the second is the reactive multiplication of connective tissue; the third is the deposit of that calcium which is produced by the organism itself The latter is promoted by the chronic inflammation brought about by the mechanical and chemical irritation of the foreign body and also by the congestion produced in the lymph paths, in the lymph node sinus" (Fig. 207)

In the later stages, the picture of a proliferative-desquamative chronic lymphangitis develops "together with stasis brought about by the dust-containing cells".

The cells containing silica are propelled on by the lymph flow, as a consequence of which first the lymph nodes of the hilus and later — partly by way of retrograde lymph flow — also the more distant lymph nodes become diseased, e.g. the submaxillar lymph nodes, the cervical,

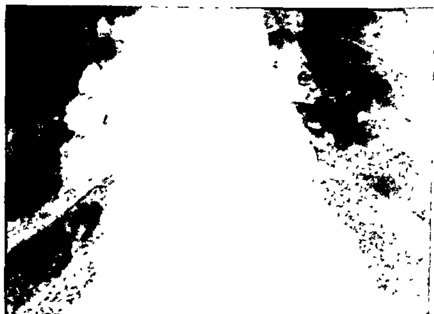


*Fig 207* Silicosis of the lung

paratracheal, sternoclavicular, mesenteric, perigastric, inguinal nodes, etc.

Dvizhkov declares that the anatomical alterations of the lung parenchyma itself commence at a time when, with the progress of silicosis, a considerable part of the efferent lymphatics is already diseased and obliterated; a mechanical insufficiency of pulmonary lymph circulation arises. "A comparison of the lesions of the lymphatics in the respiratory tracts and in the lung at an early stage of silicosis with those seen in advanced silicosis leads to the conclusion that the epithelium of the lymph vessels must have actively co-operated in the ingestion and transportation of intracellular and extracellular dust; a considerable part of it is then eliminated from the process of absorption when the disease reaches a more advanced stage: the capacity of the efferent system is considerably limited at any rate".

lung which is least dilatable and takes practically no part in the respiratory movements; consequently, it is here that the pathogens will become "trapped" most easily. This last point of view is, at any rate, more important, as it is valid not only in cases of aerogenic but also in those of haematogenic infections: also those bacteria which pass into the interstitial space from the blood capillaries are preferentially "arrested" in the apical region where the "ebb and flow" of the lymph is so sluggish.

The second problem is the question regarding the differences between the tuberculosis of children and that of adults. According to Rich (1951), *the differences are these:*

*a)* In childhood, it is not the apical region that is preferentially affected by lung tuberculosis: any part of the lung may equally be attacked.

*b)* In childhood, no fibrosis develops; the result is caseation, as a rule.

*c)* In childhood, regional lymph nodes grow larger and become caseous, while they do not grow larger in adults and contain but small tuberculous foci.

*d)* [illegible]

Acc[illegible] fact that th[illegible] the lymph flow in them is much more vigorous than in adults. We believe that, while the difference between the tuberculosis of children and adults is — partly at least — of an immunological nature, there can be no doubt that the difference between the lymphatic system of children and adults must also be regarded as an important factor, a hypothesis confirmed by the works of Soviet anatomists (Parfenova 1952; Rotenberg 1952; Strukov 1933 and Rabinovitch 1935). "In adults . . . the lymph capillaries are evidently obliterated at numerous points; . . . we can sometimes see that a portion of the peripheral lymphatic network, while filling with dye, becomes bloated and that, with continued injection, its capillaries disrupt, thus giving rise to extravasations. It avails us nothing if we try in such cases to propel the dyestuff by means of the handle of the scalpel. This being so, it is safe to assume that the obstacle in the lymph path is insurmountable" — writes Rotenberg. Strukov (1933) and Rabinovitch (1935) found that the septa of children, consisting of broad, loose connective tissue and abundantly supplied with lymphatics, grow more compact and tighter with advancing age and that, therefore, the lymph vessels in them become compressed.

There still exist many anastomotic communications between the superficial and the deep lymph vessels in the lung of children. With advancing age, these communications become gradually closed up. [illegible] deeper layers".

As to the question of *anthracosis*, Staehelin (1925) says that the boundary between "normal" anthracosis and "pneumonoconiosis anthracotica" is rather uncertain. Anthracosis is not to be considered pathological as long as it is restricted to the characteristic anthracotic pattern of the lung surface and the formation of nodules. These nodules arise from the deposit of soot particles in the lymph nodes. As a result of soot being stored in the lymph paths, connective tissue always multiplies to a certain extent. Regional lymph nodes grow larger and become sclerotic. Anthracosis becomes pathological only when it leads to desquamative and interstitial pneumonia, lymphangitis and the *obliteration of lymphatics*, whereas — in the most serious cases — the anthracotic foci, converted into connective tissue, become necrotic and disintegrate. This will occur when the occlusion of the lymphatics becomes complete and the multiplication of the connective tissue in the surroundings of the anthracotic nodes assumes such dimensions as to render the nutrition of the cicatricial tissue in the centre of the nodes insufficient. Irregular caverns will develop in such cases; surrounded by a detrital mass, these caverns are frequently connected with bronchiectases ("Phtisis atra").

Staehelin, too, shares the view that the "metastases" of pneumoconiotic phenomena may appear in different parts of the organism, not only in the natural direction of the lymph stream (right lymphatic trunk, thoracic duct), but also by way of retrograde lymph flow so that also the abdominal, cervical and axillary lymph nodes may become "infected". Certain observations point — as has already been noted — to the possibility that dust grains may occasionally gain direct access to the blood stream.

## ROLE OF THE PULMONARY LYMPHATIC SYSTEM IN TUBERCULOSIS

It is known that certain forms of pulmonary tuberculosis may also *lead to a cirrhosis of the lung. It is certain that lymphatic* obstruction is the real cause of the pathological process in these cases too. It has been mentioned that a congestion of lymph in the immobilized parts of the lung must play a role in the therapeutic effect of *pneumothorax*.

In connection with pulmonary tuberculosis, two questions have to be discussed.

The first question is the problem of a "predisposition" of the apices in the tuberculotic diseases of the lung. Although it is known today that the pulmonary tuberculosis of adults begins infraclavicularly and not apically, the craniocaudal spread of tuberculosis is nevertheless undeniable. The notion of Tendeloo (1902, 1925) is worth mentioning: the kinetic energy of the expiratory air stream is very low in the apex, especially in its paravertebral section, wherefore corpuscular particles are mostly arrested here. Still more important is the fact that lymph flow is sluggish in the apex, i. e. that part of the

parts of the lung. In so far as the infected lymph becomes so congested, the possibility of a retrograde infection of the peripheral and even more distant portions of the lung cannot be excluded (however, not against the lymph stream but beside the lymphostasis)".

This description shows that also Strukov does not postulate retrograde lymph flow but accepts the possibility of retrograde infection and thinks that in such a case several groups of lymph nodes must participate in the process; this is, however, an important circumstance from our point of view.

Relying on our [illegible] nyák and Szabó 1[illegible] retrograde lymph [illegible] succeeded in demonstrating fat by means of lipoid stains in the main efferent pulmonary lymph channels of dogs that were killed 24 hours after the ligature of the thoracic duct and the right lymphatic trunk. We interpret this phenomenon by assuming that the valves became insufficient as a result of lymph congestion so that chyle could freely flow into the pulmonary lymphatics.

## LYMPHATIC INFECTION OF THE LUNG

It is known that bacterial diseases of the tonsil may produce metastases in the lung. It used to be assumed that the pathogens penetrated directly into the tracheobronchial lymph nodes from the tonsils via the cervical trunk. Recent anatomical investigations have made it clear that communication between the respective systems of cervical and thoracic lymph vessels is very rare: the cervical trunk unites with the thoracic duct and the right lymphatic trunk only immediately before it reaches the Angulus venosus, and it also occurs that the lymph trunks empty separately into the subclavian vein. It is but rarely possible to inject a lymph node of the pleura from the direction of the cervical trunk, or a cervical lymph node from the bronchomediastinal duct. The more frequent and more probable path of infection is evidently not the direct one given by the lymphatics which would require a retrograde lymph flow also in the region of the thoracic cavity, but an indirect one: pathogens travel from the tonsils via the regional cervical lymph nodes into the cervical trunk, from here into the venous system, through this into the right heart, then into the pulmonary artery and capillaries; if, after being arrested here, they pass into the interstitial tissue, it is possible for them to be carried by the lymph flow even into the tracheobronchial lymph nodes. Owing to the special position of the lung in the blood circulation, all pathogenic agents which gain access to the lymphatics somewhere on the periphery and are then carried through the lymph paths into the venous system, must likewise arrive at the lung and its regional lymph nodes.

It is not intended to discuss here in detail the question of the spread of pulmonary tuberculosis via the lymph vascular system. This is a well-known and much-discussed subject. We refer to the works of Shtefko (1935, 1937) who attaches [illegible] s [illegible] a [illegible] the existence of a lymphogenous form of pulmonary tuberculosis but admits that the lymphatics of the lung play an important role in the course of pulmonary tuberculosis.

The question whether there exists a *retrograde stream* in the pulmonary lymphatic apparatus is connected not only with the pathology of lung tuberculosis but also with other important problems, e.g. the spread of intrapulmonary tumour metastases. The Soviet authors, Arustamova (1933) and Andreyev (1939), are of the view that under certain conditions, e.g. when the tracheobronchial lymph nodes are blocked, a retrograde lymph flow may occur in the pulmonary lymphatics. Consequently, pathogens may be driven via the lymph vessels from the central areas of the lung to the periphery. Rotenberg writes the following: "Our findings in respect of the intrapulmonary lymph flow point to the probability that — in connection with the act of respiration — lymph under normal physiological conditions flows in a centrifugal direction in the vessels that run in the deeper layers of the lung. It must be therefore assumed that retrograde lymph flow does not occur as a pathological phenomenon either. As regards retrograde lymph flow from the lymph nodes of the Radix pulmonis which may occur under pathological conditions, we do not deny its possibility but regard it as an exception. At the point whence the efferent superficial lymphatics issue, numerous valves are to be found not only in the large vessels but also in the smaller ones. Likewise a great number of valves are encountered in the lymphatics of the deeper layers, mainly in those portions which are situated outside the organs. This whole valvular apparatus represents, in our opinion, a peculiar barrier which protects the lung from the retrograde lymph flow coming from the lymph nodes of the root of the lung. Besides, if any particular group of lymph nodes is blocked, the numerous anastomoses between the lung lymph vessels make the development of collateral paths, leading to intact lymph nodes, always possible. Therefore, in the majority of those cases which are regarded as examples of a retrograde spread of tuberculous infection through the pulmonary lymph paths, what really happens is that tubercle bacilli or their toxins are spreading through the interstitial ground substance of the connective tissue by means of the mechanism examined by Ravitch-Shtcherbo or else that one is faced with a retrograde infection". The latter is described by Strukov as follows: "If several groups of lymph nodes of the radix participate in the tuberculous process, conditions will develop which must lead to a blockage of the lymph flow, as a consequence of which lymphostasis arises, first near the hilus and later also in more distant

... were ... in

lung; it was then ... emphysematous bullae, in reality *lymphangiectasis* ... *fluid* · great numbers of macrophages were visible which had phagocytosed lipid gran ... were extremely dilated. The lymph vessels forn ... engorged with fat ...

Particular interest attaches to this case not so much because of the chylous ascites and hydrothorax as on account of the chylous "pneumonia", since *chylous ascites and hydrothorax were mentioned* in the literature already centuries ago (see the corresponding chapter).

How are we to explain the appearance *of chyle in the alveoli?* We have already noted in the chapter on pulmonary oedema that drops ... in the major efferent pulmonary lymphatics ... cases of long- ... as the alveoli. In the case of the ... strange that it was in the right lung where the striking changes appeared. As is known, the lymph of the whole right lung and of the greatest part of the left lung is drained, as a rule, not by the thoracic duct but by the right lymphatic trunk so that a chylous "pneumonia" as a result of the obliteration of the thoracic duct is inconceivable unless we postulate simul ... variation where the greatest part of the lung ... duct.

A ... d his associates was published also by ...

## ROLE OF THE PULMONARY LYMPHATIC SYSTEM IN THE PATHOGENESIS OF ACUTE DIFFUSE INTERSTITIAL PULMONARY FIBROSIS

... lack of ...

... d that the matter is not one of ... *infection does play a role in the* ... iral infection may be considered. Also chemical agents were ... o account.

We recently had occasion to observe two such cases; one of the cases was diagnosed *in vivo* (Julesz and co-workers 1954). Table 67, compiled from the cases described by Callahan, Sutherland, Fulton and Kline (1952) includes also our two cases.

## THE LYMPH VASCULAR SYSTEM OF THE LUNG AFTER PNEUMONECTOMY

We have already mentioned Rotenberg's opinion (see chapter on anatomy) that investigations concerning the lymphatic system of the lung have become especially important, because surgical interventions on the lungs are now undertaken with increasing frequency. In this connection, the work of Schulze and May (1931) merits attention: it is stated there that lymph circulation becomes more vigorous in the remaining parts after a partial resection of the lung. This was proved by the observation that intratracheally administered India ink reached the regional lymph nodes of the lung more rapidly in such cases than under normal conditions.

## LYMPHANGIECTASIA PULMONALIS, CHYLOUS PNEUMONIA AND CHYLOUS HYDROTHORAX

It has already been noted that Delarue, Depierre and Roujeau (1950) had described an interesting clinical picture under the term *Lymphangiectasia pulmonalis, chylous pneumonia.*

A female patient, aged 40, felt sharp pain in the left arm 8 days post partum; within a short time oedema appeared which, beside the left upper extremity, extended also to the anterior wall of the thorax. The pain abated in 8 days, and the patient left the hospital soon afterward; a slight oedema was still existing in her left arm

Sudden dyspnoeic attacks supervened 4 months later; they appeared twice to three times during the night and resisted all kinds of therapy. The dyspnoea subsequently became chronic and the patient developed anorexia as well. The patient

2,3 g% of fat.

Approximately two years following the commencement of the disease a phlebogram was made which suggested the diagnosis of a thrombosis of the left subclavian vein. The area of this vein was then laid open· several conspicuously dilated and en-

Histological analysis revealed a mostly recanalized organized thrombus in the excised piece of the subclavian vein

The fatal consequence of this superfluous operation manifested themselves

were found in

a similar fluid.

l swollen hard

lymph nodes were observed in the mediastinum. The operated postclavicular area

| Author | Age | Sex | *Profession* | Symptoms | Duration of disease | Size of heart | *Clinical observations* |
|---|---|---|---|---|---|---|---|
| Ferrari et al. (1949) | 44 | male | ? | coughing, emaciation | 1 year | ? | ? |
| Peabody et al. (1951) | 47 | *female* | ? | dyspnoea | 2 years | ? | ? |
| Golden and Tullis (1949) | 41 | male | welder | coughing, dyspnoea, *oedema* | 6—7 years | hypertrophy and dilatation of right *ventricle* | rales |
| Golden and *Tullis* (1949) | 42 | *female* | ? | dyspnoea, oedema | ? | hypertrophy and dilatation of right ventricle | rales |
| *Rubin et al.* (1952) | 44 | female | secretary | dyspnoea, oedema | $2^1/_2$ years | 245 g; dilated Conus pulmonalis and left ventricle | rales |
| Keenland and *Smetana* (1940) | 47 | *female* | ? | haemoptoea fever | 10 weeks | 400 g; mild atrophy of right *ventricle* | rales |
| Callahan et al. (1952) | 55 | male | filling stat. service man | coughing, dyspnoea, *haemoptoea* | $3^1/_2$ months | 350 g | rales |
| *Erdélyi* et al. (1954) | 26 | male | filling stat. service man | dyspnoea, cyanosis | 15 months | 415 g; both ventricles dilated | small shadow of a miliary character (*see Fig.* 206) |
| Julesz et al. (1954) | 49 | male | employee | dyspnoea, cyanosis | 18 *months* | 450 g | rale, small, spotty *diffuse* shadow |

TABLE 67

*Acute diffuse interstitial pulmonary fibrosis (clinical picture and symptoms)*

| Author | Age | Sex | Profession | Symptoms | Duration of disease | Size of heart | Clinical observations |
|---|---|---|---|---|---|---|---|
| *amann and Rich (1944)* | 47 | *male* | worker | *coughing, dyspnoea, oedema* | 4 months | *somewhat enlarged* | *raucous respiration;* rales |
| amann and Rich (1944) | 21 | female | ? | dyspnoea, fever | 46 days | 350 g; dilated right ventricle | moist rales |
| amann and Rich (1944) | 37 | female | ? | haemoptoea | 31 days | hypertrophy and dilatation of right ventricle | occasional fine rales |
| amann and Rich (1944) | 68 | female | ? | coughing, dyspnoea | 6 months | 345 g | crepitation, basal damping |
| der et al. (1945) | 47 | male | waiter | dyspnoea | 4 months | 310 g | occasional rale |
| otter and Gerber (1948) | 33 | male | typographer | emaciation, debility | 32 weeks | 300 g; hypertrophy and dilatation of right ventricle | rales |
| eams and Harmos (1949) | 32 | male | seaman | dyspnoea | 15 months | 370 g; dilated right ventricle | rales |
| eabody et al. (1950) | 44 | female | housewife | coughing, dyspnoea, haemoptoea | 3 1/2 years | normal | negative |
| errari et al. (1949) | 63 | female | ? | coughing, dyspnoea | 4 months | ? | ? |

With regard to the fact that, at the onset of the disease, interstitial oedema is present which develops later into interstitial fibrosis, and that the above-mentioned similarity exists between the different forms of pulmonary oedema, it seems safe to conclude that *some kind of disturbance in the pulmonary lymph circulation must surely play a role in the genesis of acute diffuse interstitial lung fibrosis.* Whether this disturbance is only secondary in the sense that oedema fluid of an inflammatory or toxic origin appears in the interstitial spaces of the lung for the removal of which the lymphatic system has become insufficient, or whether we have to deal with a primary disorder of the lymph flow, has not yet been ascertained. Noteworthy is a remark made by Erdélyi and his associates in connection with their case: "It is conceivable that a lesion in the nervous system (as already mentioned, the disease was preceded by a railway accident) may have produced secondary lymphatic changes (Erdélyi and his associates are probably referring to lymphangiospasm), which led subsequently to interstitial oedema and consecutive fibrosis. Naturally, the connection of this pathological process with a probably unknown infection is likewise possible".

Vaněk (1954) described 16 cases of interstitial pulmonary fibrosis, with marked symptoms of obliterating lymphangitis in three of them

process... The final stage corresponds to the so-called cirrhosis of the lung".

The Table shows that the disease occurs in adults and that both sexes are equally affected, further that the disease lasts from 1 month to 7 years but leads to death generally within 1 to 2 years. The Table shows moreover that hypertrophy and dilatation of the right heart is demonstrable in most of the cases, a natural consequence of the multiplication of the interstitial connective tissue and the consequent obstruction of the pulmonary circulation. The occupation of the patients offers no clue to aetiology. It is perhaps noteworthy that in two



*Fig. 208.* Interstitial pulmonary fibrosis

cases (Callahan et al.; Erdélyi et al.) the inhalation of benzine played a certain part. In the case of Erdélyi, Kisfaludy, Sándor and Rácz (1954), the disease was preceded by a railway accident (Fig. 208).

We have discussed this disease because its histopathologic picture shows changes strikingly similar to those observed in the lymphoedema [illegible]

injections of quinine silicate and accompanied by lymphatic occlusion. Let us further remark that a great similarity exists also between the histopathological picture of terminal ileitis and that of interstitial pulmonary fibrosis.

We would also mention another observation of earlier authors: chyle *always contains neutral fat*, even if fatty acids have been introduced into the intestine. This is indicative of the fact that the intestinal epithelium is capable of synthetizing both fat and glycerol (Munk 1884; Radzieyewski 1868).

An interesting question of great importance arises: why is fat absorbed towards the lymph capillaries and not towards the blood capillaries? Chaikoff and his associates (1952) thought of two alternatives: it is possible that blood capillaries are impermeable to fats or, again, that the absorption of fat through the endothelial cells of the lymph capillaries constitutes an "active" process in which energy is consumed.

## "LYMPHOGENIC STEATORRHOEA"

After having discussed the above physiological data let us now consider the pathological picture of the well-known mesenteric tabes. It is commonly known that a tuberculous obliteration of the mesenteric lymph nodes and lymph vessels in children leads to a cessation of fat absorption, infantilism and atrophy. It is nevertheless strange that — when, in a given case, the aetiology of fatty stools is examined — the disturbances of lymph circulation are left out of consideration. If no icterus is diagnosed and also organic lesions of the intestinal wall — chronic enterocolitis, regional ileitis, ulcerative colitis — can be left out of consideration one is usually content with examining the pancreas: if its functioning seems to be undisturbed one simply speaks of "idiopathic" steatorrhoea. Both the tropical and non-tropical forms of sprue are essentially "idopathic" steatorrhoeas.

Let us quote a passage from Ryle (1924): "Disturbance of fat aqsorption and its concomitant complications, infantilism and tetany, occurring in the non-tropical form of sprue, are most conveniently explained by assuming an obstructive lesion of the lacteal vessels. The lesions are, presumably, of infectious origin, since the disease is acquired and not congenital." A characteristic case is adduced by Ryle in support of his concept. A young married woman who had been ill with intermittent diarrhoea since her childhood developed steatorrhoea and tetany. The clinical appearance of the disease was perfectly similar to that usually described as "idiopathic steatorrhoea". The patient was operated upon. The surgical intervention revealed a tuberculous caseation of the mesenteric lymph nodes; the mesenteric lymph channels were filled with chyle, to the point of bursting.

Bockus (1946), too, warns that we must always consider the possibility of some organic disease (tuberculosis, lues) of the mesenteric lymph nodes in cases of "idiopathic steatorrhoea", and that a disease of this kind cannot be diagnosed unless autopsy is performed.

Autopsy in cases of steatorrhoea usually fails to reveal congestion in the lacteals: this does not, according to Ryle, obviate the

# CHAPTER XV

# THE GASTRO-INTESTINAL TRACT

## ABSORPTION OF FAT

It is well known that mesenteric lymphatics absorb the major part of fat from the intestines; this applies to cholesterol also. Fats, passing through the epithelial cells of the intestines, can be followed in histological preparations as far as the central lymphatic channels. It was demonstrated by Zawilski in Ludwig's institute (1876) that most of the ingested fat got into the thoracic duct from the intestine. The portal vein absorbs only a negligible part of the fat, although — according to certain authors — the concentration of fat is always higher in the portal blood than in the jugular vein (d'Erico 1906/7).

Earlier authors regarded absorption of fat from the intestine as a slow process. Eckstein (1925), for example, administered olive oil to dogs and found that within 12 hours not more than 21 per cent appeared in the thoracic duct. The absorption of oleic and palmitic acid was still slower. Similar results were registered by Little and Robinson (1941). By using a different technique, Bloom et al. (1950) obtained quite other results: they cannulated the thoracic duct of unanaesthetized rats with Bollman's method and studied the absorption of radioactive fat. They found that 81 to 95 per cent of the fatty acid labelled with $^{14}C$ had been absorbed within 19 to 24 hours and that 70 to 92 per cent of the absorbed quantity had appeared in the thoracic duct lymph. It is very likely that the remaining 8 to 30 per cent was not absorbed through the blood vessels but found its way to the circulation via other lymphatics (right lymphatic trunk, etc.). The low figures obtained by earlier authors are in all probability due to their having experimented on anaesthetized and immobile animals. In another group of experiments, Bloom and his collaborators (1951) administered palmitic acid labelled with $^{14}C$ to the animals, and succeeded in recovering 96 per cent thereof from the thoracic duct. It was furthermore discovered (Chaikoff et al. 1951) that not only fatty acids with an even number of carbonic atoms, i.e. natural fatty acids were absorbed into the lymphatics: also fatty acid chains labelled with radioactive carbon and composed of 15 carbon atoms, were found to have been absorbed; 78 to 90 per cent of the introduced and 89 to 93 per cent of the absorbed $^{14}C$ appeared in the thoracic duct in an unchanged, unesterified form. Essentially, the same can be said of cholesterol. Although not more than 22 to 49 per cent of the labelled cholesterol was absorbed, 94 to 100 per cent of the absorbed quantity was discharged through the thoracic-duct fistula, half of it in a free, the other half in an esterified form (Chaikoff et al. 1952).

## REGIONAL ILEITIS

The postmortem picture of regional ileitis is known to be characterized by a marked thickening of the intestinal wall accompanied by a constriction of the lumen and ulceration of the mucous membrane in the affected intestinal segment. Also characteristic is the oedema- ... belonging to the diseased area, and ... nodes: their diameter reaches, according to Bockus (1946), 0·5 to 2 cm. Histologically, oedematous swelling of the submucosa, accompanied by a hyperplasia of the lymphatic tissue elements and an obliteration of the lymphatics, i.e. by *obstructive lymphangitis*, is observable in the initial phase of the affection (Schepers 1945; Pratt and Ferguson 1947; Smith 1941; Bargen and Dixon 1935). While, according to Blackburn et al. (1939), lymphatics are occluded by masses of lymphocytes, Warren and Sommers (1948) ...

... nodes. Granulomatous proliferation with giant cells is encountered in the lymphatics. The granulomata undergo a gradual hyaline degeneration, usually without necrosis. Beside these phenomena, desquamation of the lymphatic endothelial cells is an additional factor that contributes to the obliteration of the lumen. The same conclusion was reached by Rappaport and Burgoyne (1948) as also by Hadfield (1939). Hadfield emphasizes that no acid-fast bacteria are demonstrable in the granulomata. These alterations are later followed by fibrosis, elephantiatic degeneration and ulceration of the tissues, and the subsequent ulceration of the mucous membrane. This histological picture, together with the circumstance that the disease mostly occurs in those portions of the intestine which possess a specially well-developed lymphatic apparatus, has of course given rise to the presumption that pathological alterations of the mesenteric lymph vascular system must play a decisive role in the aetiology of regional ileitis. That the alterations in question have a decidedly segmental character and the boundary of the diseased segment is sharply defined speaks in favour of the theory.

Of significance were the animal experiments of Reichert and Mathes (1936) which furnished a decisive proof of the fact that regional ileitis is a disease which belongs to the sphere of lymphatic pathology. Essentially, these authors performed the same experiment on the intestine as had been made by Drinker and his collaborators on the hind leg of animals: they blocked a group of mesenteric lymphatics by the injection of sclerosing ...
oedema developed in that p...
by the blocked lymphatics: t...
fibrosis, cicatrization, followed by the ulceration of the mucosa,

possibility that congestion occurred after the ingestion of fat *in vivo*.

Relying on the analogy offered by phlebohypertension, i.e. cardiac decompensation, we are in full agreement with this theory: also in such cases are lymphatics distended *in vivo* and promptly emptied after death. *Another point, too, should be noted: organic* disease of the mesenteric lymph nodes and lymph vessels is in our terminology equivalent to *a mechanical insufficiency of the lymph circulation*. It could well serve as a working hypothesis for further investigations — that there may exist also another kind of insufficiency of the mesenterial lymphatics. We are thinking in this connection of the different forms of *absorption insufficiency*. It might, for instance, be assumed that the intestinal lymph capillaries undergo a pathological alteration. It is also possible that intestinal epithelium has — perhaps by having lost its power of esterification — become impermeable to fats; one must further consider the possibility of reduced peristalsis (a phenomenon that has actually been radiographically demonstrated in similar cases), as also that of a cessation of the autonomous movement of the villi; that movement is the motor of lymph propulsion is true in the intestines as well. We pointed out earlier that there are data which point to a rhythmic contraction of the mesenteric lymph vessels. Webb (1933), for example, counted 15 to 18 contractions per minute in the rat, and this frequency induced him to repudiate the possibility of a movement transmitted by respiration or arterial pulsation. However, the possibility still remains open that one has to deal with a movement transmitted by intestinal peristalsis which has a similar frequency. An argument against such possibility is furnished by Webb himself (1937) who succeeded in influencing the rate of contraction of the chyle vessels by the local administration of various drugs. Rhythmic beats ceased and the lymphatics contracted under the action of pitocain and pitressin (pH 3·77). Pilocarpine, if administered in a dilution of 1 : 1000 (pH 5·10), promptly stops rhythmic contractions. Adrenaline in a dilution of 1 : 10 000 (pH 6·55) has the effect of augmenting the rate of contraction and of dilating the lymphatics. Barium chloride (dilution 1 : 1000, pH 7·6—7·8) influences the rhythm of contractions in a similar manner but causes spastic contractions. Nembutal, in a dilution of 1 : 100 (pH 9·02) produces no effect on the amplitude of the contractions but increases their frequency. All this makes it clear that the rhythmic contraction of the chyle vessels is directly affected by various drugs and toxins and that their action is strongly influenced by the concentration of H-ions. It is also conceivable that fatty stools and the insufficiency of intestinal lymph circulation may arise through the cessation of normal contractions. There are undoubtedly many problems in this field which await further elucidation, but it is certain that the lymph vascular system is a factor that must in no case be disregarded.

i.e. the same alterations as occur in regional ileitis, ensued in the oedematous area.

It should be noted that a combination of lymphatic blockade with the intravenous injection of bacteria, while producing no influence on the nature of the alterations, aggravated them quantitatively. That lymphoedematous areas are especially susceptible to infections has already been noted in connection with elephantiasis.

Although Sinaiko and Necheles (1946) failed to produce, by the ligation of the mesenteric lymphatics, such chronic alterations as would have led to ulceration, the above considerations are nevertheless convincing enough for us to explain the aetiology of ileitis by assuming



*Fig. 211.* Chronic granulomatous lymphangitis of stomach. Thrombus in the dilated lymph vessel (Szinay and Szeker 1956)

that the primary pathogenic factors are mesenteric lymphadenitis and lymphangitis which, for some reason, instead of healing without leaving a trace, heal with a cicatricial occlusion of the lymphatics: this is enough for the development of the well-known grave pathological changes.

Rockey (1933) observed the oedematous thickening of the ileum in four cases of acute mesenteric lymphadenitis. Acute mesenteric lymphadenitis and acute ileitis are regarded by certain authors as one and the same disease (Jackson 1937). It should be noted that it



*Fig. 209.* Chronic granulomatous lymphangitis of stomach. Granuloma in the dilated lymph vessel (Szinay and Szeker 1956)



*Fig 210.* Chronic granulomatous lymphangitis of stomach. Granuloma in the dilated lymph vessel (Szinay and Szeker 1956)

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has *never been proved in human pathology that* acute *ileitis* can turn into true chronic regional ileitis.

Infantile tuberculous infection with the bovine type of bacteria is regarded by some authors as a factor that gives rise to a cicatrization of the mesenteric lymph vessels and lymph nodes. The fact that groups of giant cells are encountered in lymphoedematous areas and diseased lymphatics seems to support this view. It should, however, be remembered that nobody has ever demonstrated tuberculosis bacilli in connection with regional ileitis.

Probstein and Gruenfeld (1936) pointed out that the onward movement of the intestinal contents before the ileocoecal valve is sluggish even under normal conditions and that, besides, the mucous and submucous lymphoid tissue is much more developed in this area than elsewhere in the intestinal tract. These two factors, between them, render this area especially susceptible to the absorption of bacteria and the contraction of infectious diseases. To this should be added the experimental observation that, after the injection of gelatin into *the mucous membrane of the colon or the ileum, no filling* could be obtained beyond the cusp of the valve. The area in question is, thus, especially endangered as regards the "adhesion" of infections.

Rényi-Vámos and Szinay (1957) postulate a regeneration of the lymphatic apparatus in cases of regional ileitis.

It is commonly known that regional enteritis may occur not only *in the ileum but at any point of the gastro-intestinal tract. In a case,* described by Ross (1949), there were, for instance, similar alterations in the small intestine, the large intestine and the stomach. It is held by some recent authors that some of the cases of *gastric cirrhosis* — the modern term for *Linitis plastica* — represent the final stage of the gastrically localized form of regional enteritis. That not all cases of *Linitis* plastica are necessarily of a carcinomatous *nature* and that some of them are of an inflammatory origin was emphasized by Krompecher as long ago as 1910. He ascribed the genesis of these lesions to chronic venous congestion with consequential oedema and with possible inflammatory phenomena, and declared that all these led finally to fibrosis, *sclerostenosis*. Let us add that as long ago as *1893 Bouveret* advanced the theory that chronic *indurative gastric* oedema was often of lymphatic origin. In a case, published under the title "Chronic granulomatous lymphangitis of the stomach wall", Szinay and Szeker (1955) described exactly such lymphangitic alterations and such a formation of granulomata in the lymphatics (Figs. 209—211) as are characteristic of regional enteritis. The knowledge of this *pathological picture is of practical value in differential diagnosis* since it helps to distinguish it from gastric carcinoma (Mester 1952).

As regards the therapy of regional enteritis just as in advanced cases of elephantiasis of the extremities a radical extirpation of the diseased area is the sole possible remedy, so must recourse be made to *resection* in extreme cases of regional enteritis.

## ULCERATIVE COLITIS

As in the case of regional ileitis, a damage of the intestinal lymphatic apparatus probably also plays a role in the pathology of ulcerative colitis, although the histological picture is not quite as characteristic of lymphoedema in the latter. The mucosa and submucosa of the affected intestinal portion are found to be oedematous, hyperaemic, and the wall of the intestine thickened in the initial phase of ulcerative colitis. The muscularis mucosae, too, is oedematous, though to a lesser extent. The mucous membrane subsequently becomes necrotic and ulcerative.

Poppe (1941), after blocking the lymphatics of the mesentery in the ileocoecal area by the injection of sclerosing substances, found that, besides oedema, inflammatory phenomena, lesions of the small blood vessels, haemorrhages and, finally, necrosis and ulceration of the mucous membrane developed in the coecum. Similar damages can be provoked in the rectum as well, and it is emphasized by Poppe that this is achievable by a mere ligature of the lymphatics, without intravenous injection of bacteria. Intravenous injection of quite a number of bacteria without ligating the lymphatics produced, on the other hand, nothing more serious than acute generalized enteritis and failed to cause chronic ulcerative inflammation of the intestinal tract in any of the experiments.

Bockus, too, regards Poppe's experiments as highly significant and suggests that, in human pathology, this may be the pathomechanism of ulcerative colitis caused by lymphogranuloma venereum.

That a correlation exists between ulcerative colitis and the nervous system is commonly known. Could not also a neurogenic lymphangiospasm play a certain role in this connection? A functional lymphatic obstruction of but a few days' duration may quite suffice for the development of anatomical alterations.

## APPENDICITIS

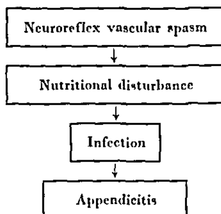
Terming it "*primary lymphangitis of the appendix*", Borchard described in 1928 a special form of acute appendicitis. He regarded the clinically unusually high degree of fever and strikingly few local symptoms as characteristic features of this form of appendicitis. Appendectomy is followed by a gradual abatement of temperature. Laparotomy reveals but a small amount of serous exudate in the abdominal cavity. Examined macroscopically, the appendix appears at first sight intact, but the regional lymph nodes are swollen, and narrow red streaks run from the appendix to the lymph nodes. Histological examination shows lymphangitis. Borchard emphasizes that both appendix and the swollen, inflamed regional lymph nodes have to be resected if we want to prevent possibly grave complications. It may happen that the lymph nodes begin to suppurate

and, disintegrated, give rise to purulent peritonitis; it also happens that the lymph nodes become cicatrized and shrunken which may lead to subsequent complaints.

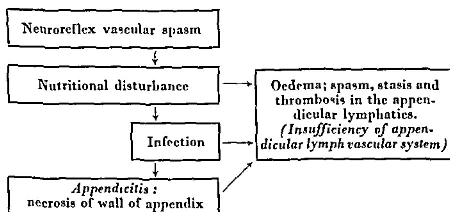
In "common" appendicitis, on the other hand, the appendicular lymphatics become usually thrombotic in an early phase of the disease: a lymphatic spreading of the infection towards the regional lymph nodes is thus prevented so that the process remains — for the time being, at least — localized to the appendix. It is anyway worth while pondering over the question as to what must happen to an organ if its lymphatic system becomes thrombotic and obliterated at a time when a simultaneously existing inflammation strongly increases capillary filtration. We shall have occasion to see that a hydronephrotic kidney may necrotize if its lymphatics are tied off and that an obstruction of the hepatic lymphatics aggravates the condition of the cholangitic liver. We are convinced that, among other factors (alteration of blood vessels, infections, neurogenic factors), obliteration of the lymph circulation surely plays a role in the development of gangrene in the inflamed appendix.

Speaking of appendicitis, we have to mention the investigations of Fischer and Kaiserling (1936): they succeeded in producing allergic lymphangitis by reinjection into a lymph vessel in sensitized organisms. Sterile lymphangitis developed in this lymph vessel. If, for instance, it was into the lymphatics of the gall bladder that the reinjection was made, lymphangitis developed in these lymphatics, and the inflammation involved also the wall of the gall bladder: the process resulted in cholecystitis. By reinjections into one of the appendicular lymphatics it was possible for Fischer and his co-workers to provoke regular appendicitis with all its consequences. This is, of course, not the mechanism through which appendicitis arises in human pathology; if, however, appendicitis has developed for some reason, the inflammation spreads, in a secondary manner, to the lymph vascular apparatus of the appendix. Fischer's experiments are successful reproductions of Borchard's primary appendicular lymphangitis.

Norenberg-Tcharkviani's work (1954) is well in line with these considerations. This author succeeded in provoking "segmental" appendicitis by ligating the blood vessels which run to the appendix. He concludes from these experiments that a neuroreflex mechanism, which leads to vasoconstriction in the wall of the appendix, is the primary factor in the pathogenesis of appendicitis. This causes trophic nutritional disturbances which, then, pave the way for infections:




Norenberg-Tcharkviani's theory disregards the pathological change of the appendicular lymphatic apparatus although such changes were actually observed in the course of his experiments: he described pronounced stasis not only in the arteries and veins but in the lymph- [illegible] by the inclusion of lymph-circulatory insufficiency:




We want to emphasize that we have performed no experiments with a view to studying the pathogenesis of appendicitis so that we are not in a position, nor do we intend, to take a stand either for or against Norenberg-Tcharkviani's theory or any other theory concerning the development of appendicitis. One might also regard infection as the primary phenomenon which gives then rise to vas-

and, disintegrated, give rise to purulent peritonitis; it also happens that the lymph nodes become cicatrized and shrunken which may lead to subsequent complaints.

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These investigations of Fischer and his collaborators have more than merely theoretical significance. Allergic lymphangitis and consequential sympathicoganglionitis hardly occur in human pathology, but it is well-known that an inflammation of the abdominal organs, whatever its cause, spreads over to the lymphatics of the

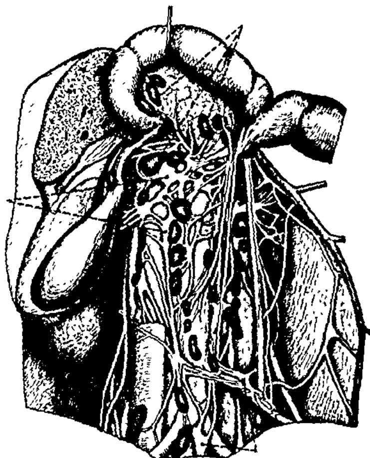


*Fig. 212.* Topography of the intraabdominal lymph nodes and sympathetic ganglia (after Kiss)

affected organs: lymphangitis thus arisen may then damage the adjacent ganglia in quite the same manner as was seen in Fischer's animal experiments. To be aware of this possibility enables us to consider the direct correlations between diseases, inflammatory processes and the nervous system from a new point of view.

Of great significance in this connection is one of Zhdanov's recent works (1959b). After injecting Gerota-blue into the intestinal wall,

cular spasm, nutritional disturbances, lymphatic thrombosis, etc. All we wanted to show was that the appendicular lymphatic apparatus and its pathologic changes play an important role in the pathology of appendicitis.

## LYMPHOGENIC SYMPATHICOGANGLIONITIS

The abdominal sympathetic ganglia and the large abdominal lymph nodes are juxtaposed at many points, a phenomenon that was first pointed out by Kiss (1930, 1931, 1951), one of whose illustrations we find reproduced in Rouvière's monograph (Fig. 212). It was likewise Kiss who emphasized that the anatomical connections between the sympathetic ganglia and the lymph vascular system could not fail to produce also clinico-pathological effects: inflammatory processes and lymphangitides might directly — *per continuitatem* — spread over to the ganglia. He writes this: "Our experiments have made it evident why so many organic injuries (damage of thoracic lymph nodes above the vagus, gastric ulcer; diseased abdominal and pelvic lymph nodes, disturbance of adjacent organs) can be caused by infectious and tumorous diseases of the lymph nodes in the body cavities. According to a personal communication made by the Rumanian professor Hatigean, he found not less than 13 different serious post-operative abdominal damages following appendectomy. No sooner did he catch sight of the illustrations accompanying our publications than the correlation between his observations and the abdominal *lymph nodes infected by the way of appendix became clear to him.* The lymph nodes wedged between the fibres of the aortic plexus and those seated on the coeliac ganglion may also become infected by the appendix. It is especially the latter which are able to provoke disturbances in many organs, since the coeliac ganglion is the largest sympathetic centre of the abdominal cavity. Earlier clinicians regarded this large ganglion as the brain of the abdomen."

There exists a *direct connection* between the sympathetic ganglia and the lymphatic system since lymph capillaries arise from the ganglia (Rouvière 1929; Troitsky 1930; Orts Llorca and Botár 1932). The animal experiments of Fischer and Kaiserling (1936) proved the correctness of Kiss's concept beyond any doubt. As has been noted in the chapter on appendicitis, Fischer and his associates had succeeded in producing allergic lymphangitis by intralymphvascular reinjections to sensitized animals. They were thus able to induce lymphogenic-allergic appendicitis and cholecystitis. It emerged in the course of these experiments that the sterile inflammation did not localize itself to the lymphatic apparatus but spread over to its

autonomic nervous system is assured not merely by the vicinity of the lymph nodes and the sympathetic ganglia and further by the proper lymphatics of the sympathetic ganglia: such direct communication between the two structures exists peripherally as well. It would, *of course, be desirable if Zhdanov's results, obtained by* means of injections, were confirmed also by other methods.

There is also the theory advanced by Fischer and Kaiserling (1936) according to which abdominal lymphangitis may involve the cerebral nerves. They claim to explain the "vertigo e vesica fellea laesa", described by earlier authors, by the assumption that, in cholecystitis, the inflammatory process spreads first to the lymphatics of the gall bladder, *then to the efferent lymph vessels and finally to the vagus.*

## SIGNIFICANCE OF THE LYMPH VASCULAR SYSTEM OF THE STOMACH IN THE PATHOLOGY OF GASTRIC ULCERS

It is proposed to give in this chapter a brief recapitulation of what has already been said about the lymphatic apparatus of the stomach. Rényi-Vámos and Szinay studied the dilated lymphatics of ulcerous stomachs in order to become familiar with the topography of the gastric lymphatics. *They found the environment of acute gastric ulcers to be oedematous. Recent experiments performed* at the Medical University of Szeged (Borbola, Bikich and Faredin 1955) yielded evidence to show that histamine plays a role in the development of this kind of oedema. The situation in this respect is rather similar to that encountered by Babics and Rényi-Vámos in obstructed kidneys: histamine, accumulating in the parenchyma, provokes increased permeability of the blood capillaries.

Protein-rich fluid passes into the interstitial space when the permeability of blood capillaries is augmented. We have quoted many *examples to show that, when this happens, it depends on the lymph vascular apparatus whether the whole capillary filtrate is* carried off or if part of it remains in the organ which gives then rise to the development of oedema.

The lymph vascular apparatus of the stomach becomes insufficient in cases of gastric ulcer. Rényi-Vámos and Szinay think that this insufficiency is of a dynamic character. They base this assumption on the observation that, apart from a few cicatricial lymph nodes, the majority of the lymph nodes contained distended sinusoids and the efferent lymphatics were grossly dilated.

We are quite in agreement with the view that, essentially, one is here dealing *with dynamic insufficiency. It might be assumed,* but not *proved, that dynamic insufficiency is perhaps associated with some form of absorptive insufficiency and that possibly also* lymphangiospasm is present.

he examined the duodenal lymphatics of adults and children on transparent preparations and found that the dye, injected into the layers of the intestinal wall, stained not only the muscular and subserous plexuses of the lymphatics but filled also a fairly large area in the system of perineural spaces of Auerbach's intramuscular autonomic nerve plexus. "The perineural spaces, lymph capillaries and primordial efferent lymphatics in the subserous, muscular and submucous layers of the duodenum filled simultaneously. The filling of the perineural spaces is, as a rule, more complete than that of the lymphatics."

Therefore, the perineural spaces of Auerbach's plexus are — according to Zhdanov — in communication with the intramuscular lymph capillaries: the latter may be said to arise from the former. That they belong to the category of lymph capillaries is borne out not only by their morphological characteristics, their calibre and the arrangement of their connections with the lymphatic network but also by the fact that their continuation in the efferent lymphatics can be clearly traced in the preparations as far as the efferent lymph channels which leave the intestinal wall and empty into the pancreaticoduodenal lymph nodes.

Zhdanov affirms that "...the perineural spaces stain, as a rule, more markedly with blue dye than the lymph capillary or lymph capillaries that arise from them. It can be observed that more dyestuff is contained in the perineural spaces than in the lymph-capillary network. We are convinced that, first, the dyestuff fills up the system of perineural spaces, passes then into the lymph-capillary network through the existing communications, to reach at last the efferent lymphatics...

Doubts may arise, especially when one is inspecting two-dimensional microphotographs, as to whether the origin of the lymph capillaries from the perineural spaces is not a mere illusion, i.e. whether it is not the blind end of a lymph capillary lying in another plane which is projected into the perineural space. Such doubts are, however, dispelled as soon as one looks at the connections between perineural spaces and lymph capillaries through a binocular magnifying lens or a stereoscopic microscope and turns the micrometer screw. Doing so, one sees clearly that we are dealing with true connections between the perineural spaces and the lymph capillaries and not with the blind end of lymph capillaries projected from another plane into the perineural spaces.

Without very thorough histological investigations it is rather difficult to tell the nature of the said connections, i.e. to decide whether an open communication exists or if the suspended particles have the power to penetrate through the closely-set endothelium of the lymph capillaries and the perineural spaces."

These findings of Zhdanov are indicative of the fact that a close communication between the abdominal lymphatic system and the

# CHAPTER XVI

# THE LIVER

Looking at Fig. 42 which shows the network of lymphatics around the hepatic vein, we cannot fail to conclude that this vast meshwork of tubes must, on account of its very size, have a significant task in hepatic function. We shall see that this is really so: the hepatic lymph apparatus plays a decisive role in the physiology and pathology of the liver.

## FLOW AND COMPOSITION OF LIVER LYMPH

The determination of the volume of lymph flowing in the hepatic lymph vessels encounters great difficulties. Bollman and co-workers (1948, 1950) were the first to succeed in cannulating a lymphatic coming from a lymph node in the dog; the other efferent lymphatics were ligated and also the lymph vessels arising from the duodenum and pancreas and draining into the same lymph node were closed. With these precautions, lymph flowed continuously through the cannula, usually for 24 hours but sometimes as long as 2 or 2½ days. On an average, 280 ml of lymph were so collected per day. Bollman and his associates tied a cannula into the thoracic duct as well: the amount of lymph collected through this route was 700 ml/day.

We suggest that this amount of 280 ml should be regarded as a minimum and think that the quantity of lymph produced by the liver is considerably more. This opinion relies on the following considerations:

It is known — and we have had occasion to verify this on our own material — that, in the dog, the hepatic lymph channels run not only caudally but also cranially, along the hepatic veins. This is the reason why the lymphoedema produced by a ligation of the lymph nodes in the Porta hepatis was less extensive in dogs than in cats: all, or the overwhelming majority, of the efferent hepatic lymph vessels in the latter have a caudal course. Bollman and his associates tied a cannula into an efferent lymphatic in the lymph node of the Porta hepatis and ligated all the other efferent lymph vessels. It seems probable that the cannula, which had a narrow lumen, was inadequate for a quantitative drainage of the entire volume of lymph: this led to increasing congestion and, after 24 hours, to coagulation so that the flow became more and more sluggish. In these circumstances it was possible for the cranially running efferent lymphatics, which have ample anastomotic communication

It has been noted that whenever protein-rich fluid becomes stagnant in an organ, danger of fibrosis and sclerosis arises. Rényi-Vámos is justified in supposing that lymphatic insufficiency of the stomach may be a factor responsible for the development of gastric ulcers.

While accepting this theory, we want to emphasize that neither do we nor does Rényi-Vámos regard the insufficiency of the gastric lymph vascular apparatus as the sole factor responsible for the cicatrization of chronic ulcers. As a functional change in the nervous system plays a decisive role in the origin of gastric ulcers, so does the nature of such functional change decide whether a gastric ulcer will heal without leaving a trace or become chronic and cicatrized. The healing of ulcers is undoubtedly accompanied by a cessation of pathological histamine-production and the consequent decrease of excessive blood-capillary permeability: the possibility of an absorption of the oedema around the ulcer and of the protein products of the tissue necrosis, i.e. the possibility of a "restitutio ad integrum" in the gastric parenchyma, depends, however, on a faultlessly functioning lymphatic apparatus. If — for some reason, e.g. neurogenic lymphangiospasm — the lymphatics of the stomach fail in their function of fluid transport, no remission of the pathological phenomena can take place.

the daily amount of liver lymph as too low; we think that, actually, the whole volume of the organism's circulating plasma passes through the lymphatic system of the liver within 24 hours. This significant fact has already been discussed previously.

Brinkhous and Walker (1941), experimenting with dogs, examined the prothrombin concentration of the lymph in the thoracic duct, the femoral lymphatics and the liver. Taking the prothrombin level of the blood plasma as 100 per cent, they established the following values: femoral lymph, 7·6%; thoracic-duct lymph, 50·7%; liver lymph, 93·2%. It would seem as if the high prothrombin level of the hepatic lymph was due to the prothrombin formed in the liver: against such hypothesis argues the experiment in which a colloidal dye, vital red, was intravenously injected with the result that its distribution between plasma and liver lymph was the same as that of the plasma proteins. This convinced Brinkhous and Walker that a great permeability of the sinusoids to colloidal dye and plasma proteins rather than the production of prothrombin in the liver was the factor responsible for the high prothrombin level of the hepatic lymph.

Kuhn and Hildebrand (1951) found that the protein content of the liver lymph amounted to 70 to 80 per cent of the protein content of the plasma in the dog, and to 90 per cent in the cat. They also demonstrated that intravenously administered collidon (a synthetic colloidal substance used also as plasma substitute, mol. weight 114 000) and bovine globulin appeared in the liver lymph in a high concentration. We observed in our experiments that intravenously injected dextran (mol. weight 50 000) reached in the liver lymph 86 per cent of the plasma concentration within two hours. During the same time, the concentration of Evans blue, which is bound by serum albumin, rose in the liver lymph to 71 per cent as compared with its concentration in the plasma (Szabó, 1954).

Electrophoretic comparison proved the average value of the AG-quotient to be 1·01 in the serum and 1·6 in the liver lymph.

As far back as *1894* Starling suggested that, *functionally*, the blood capillaries of the normal liver behaved like blood capillaries in other regions of the organism in inflammation, a theory shared by Krogh (1929) and With (1949). Seeing this, Kühn and Hildebrand ask whether it is still justified to speak of a serous inflammation of the liver in the sense of Rössle (1944) and Eppinger (1949).

Though the raising of this problem was perfectly reasonable we want to emphasize that the concept of Kühn and Hildebrand and other authors who regard liver lymph and the liver's protein-rich "tissue fluid" as identical is quite erroneous. It is rooted in Starling's (1896) and Drinker's theory that lymph and interstitial fluid are one and the same fluid.

We cannot but repeat that the fact that lymph, strikingly rich in protein, is streaming from a certain area does not, *in itself*, infallibly

with the periportal lymph channels, to transport lymph from the liver without disturbance.

Bollman and his associates, after having injected T-1824 into the hepatic substance, found that only the liver lymph stained blue while the lymph in the thoracic duct remained unstained. They concluded that they had been successful in collecting the liver lymph quantitatively, and did not consider the possibility that the lymphatics which run beside the hepatic vein might empty into the right lymphatic duct, the lymph of which they omitted to take into account.

Nor can we differently evaluate the other observation of Bollman and his collaborators: the bilirubin content of the liver lymph increased rapidly after the ligation of the common bile duct, whereas the rise of the bilirubin level in the thoracic-duct lymph occurred later only, at a time when it was rising in the blood as well.

If we add that Bollman and his co-workers observed a 80 per cent increase in the volume of liver lymph after activity and one of 75 per cent after meals, we can safely say that the magnitude of hepatic lymph flow is of a high order, indeed, one which is more or less on a level with that of the kidney. This is, by the way, quite in harmony with the fact that the blood supply of the liver is about the same as that of both kidneys together.

It has been mentioned in the general part of this work that authors are unanimous in declaring that liver lymph contains more protein than the lymph of any other organ. The first report in this respect is that of Starling (1909) who found that the protein level of the liver lymph approximated that of the plasma. Field, Leigh, Heim and Drinker (1934) examined the protein concentration of the liver lymph in four dogs and established its mean value at 5·32 g %, while the protein content of the serum was found to average 6·34%. McCarrell, Thayer and Drinker (1941) found that, in the cat, protein level was approximately the same in liver lymph and serum alike. Its value was in one of their animals, for example, 5·29 g % in the serum and 5·31 g % in the hepatic lymph. The qualitative composition of the proteins, too, was found to be the same in serum and liver lymph: the concentration of albumin in the serum was 3·37, that of globulin 1·92 g %, against 3·39 and 1·92, respectively, in the liver lymph. The mean values derived from individual figures established in ten cats were as follows: total protein in liver lymph 5·58, total protein in serum 5·47 g %. Brinkhous and Walker (1941) also found that the liver lymph contained approximately as much protein as the serum.

According to Grindlay, Cain, Bollman and Flock (1948), Nix, Mann, Bollman, Grindlay and Flock (1951), the protein level of the liver lymph is about 5/6 of that of the plasma. Bollman (1951a, b) suggests that about a half of the organism's total plasmaproteins streams through the lymph channels of the liver every day. We have already mentioned that we regard Bollman's data concerning

the daily amount of liver lymph as too low; we think that, actually, the whole volume of the organism's circulating plasma passes through the lymphatic system of the liver within 24 hours. This significant fact has already been discussed previously.

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We cannot but repeat that the fact that lymph, strikingly rich in protein, is streaming from a certain area does not, *in itself*, infallibly

prove that the blood capillaries of that area are particularly permeable, for — as has been noted previously — lymph and tissue fluid are not identical.

When, in our own experiments, hepatic lymphoedema was induced by the ligation of the liver's efferent lymphatics (see the anatomical chapter) it could be observed that Disse's spaces were filled with a protein-rich fluid: since the sinusoids suffered no damage in our experiments it must be concluded that, in normal conditions, there exists a constant flow of protein-rich fluid across the wall of the sinusoids towards the hepatic lymph capillaries through the Disse's spaces.

## SEROUS INFLAMMATION OF THE LIVER AND THE PROBLEM OF HEPATIC CIRRHOSIS

The concept of serous inflammation is known to have been formed by Rössle in 1944. He claims that, under normal conditions, the fluid transuding from the blood capillaries to the interstitial space is practically devoid of protein. That negligible amount of protein which still escapes into the interstitial tissue is partly carried away by the lymphatics and partly taken up and "digested" by the cells of the connective tissue. It has been noted in an earlier part of this work that Eppinger, after poisoning animals with allylformiate, allylamine or pyrrole, observed the appearance of a "serous" inflammation of the liver and other organs, a condition characterized clinically by a grave shock, thickening of the blood, increase in the number of erythrocytes with unchanged protein level, and histopathologically by a tremendous dilatation of Disse's spaces, sometimes a dissociation of the liver cells, haemorrhages, and — in extreme cases — by a complete disintegration of the hepatic structure. Eppinger suggests that one has to deal with a pathologically increased permeability of the hepatic capillaries and sinusoids: while, under normal conditions, practically protein-free capillary filtrate passes into the interstitial space, it is *plasma* which in these cases escapes from the capillaries (blood becomes more concentrated!); Disse's spaces are gorged with this plasma, and increased hepatic lymph [illegible] is removed [illegible] the oedema [illegible] n of oxygen from blood capillaries to cells result in parenchymal damage. While the necrosis of liver cells is regarded as the initial phase in the pathology of hepatic cirrhosis by other authors, it is — according to Eppinger — always preceded by a dilatation of Disse's spaces, the escape of plasma through the sinusoid walls. Congestion of protein in the *interstitial space leads then to the appearance of new* connective tissue, that is, to cicatrization.

It is suggested by Eppinger that the *limited proteolytic power of the liver cells and not the insufficiency of lymph circulation* is the decisive factor in protein congestion. We find the following passage in his work "Permeabilitätspathologie": "Hepatic lymph circulation cannot be significant for intraacinic processes as the liver *parenchyma proper contains no lymphatics*". *He holds that the* organism must possess the power to take up the fight against the "Albuminurie ins Gewebe". The lymph flow is, according to Eppinger, able to remove part of the escaped protein, the major part of the task devolves, however, on the liver cells and the intramural mesenchyme which — presumably by way of fermentation — have the faculty of protecting the parenchyma from the deleterious consequences of the escaped plasma. The so-called turbid intumescence and what is generally called compensation of the serous exudation are, according to Eppinger, visible manifestations of this process. He thinks the liver cell is probably able to take up protein that has escaped into the interstitial space and to forward it decomposed towards the interstitial fluid after some time; presumably also the mesenchymal elements (Kupffer cells and reticular fibres) take an active part in the decomposition of protein. A fluoroscopic microphotograph in Eppinger's book is a very convincing argument in favour of his statement that hepatic cells take in protein in cases of inflammation. When, *in the course of our experiments* (Földi, Rusznyák, Szabó and Donáth 1951), hepatic lymphoedema was induced in cats we, too, were able to observe by means of the fluorescence microscope that not only the Disse's spaces were filled with *protein-rich fluid but that also the liver cells themselves showed* signs of protein imbibition.

Relying on the evidence of our investigations, discussed in detail in the chapter dealing with the liver's anatomy, we are in perfect agreement with Eppinger that the hepatic parenchyma proper contains no lymphatics and that it is not penetrated by lymph vessels; that they are nevertheless of decisive importance for hepatic lymph circulation and that they transport a very great amount of protein and fluid is abundantly proved by the results of our experiments in which a blockage of hepatic lymph flow led to the prompt development of a significant lymphoedema and the dilatation of the Disse's spaces.

In human pathology, a picture strikingly similar to that of serous inflammations observable in animal experiments is produced by infectious hepatitis, beriberi, Graves' disease, etc. (see the anatomical chapter).

That the impletion of the Disse's spaces with protein-rich fluid in hepatitis indicates not merely increased sinusoid permeability, i. e. increased production of fluid, but a disturbance of the transport mechanism as well, was pointed out by Eppinger himself: he says that, in hepatitis, we are faced with a "stasis" of the intrahepatic

fluid circulation. He stated furthermore that, in cases of acute hepatitis, dilated lymphatics in the liver were of frequent occurrence.

Worthy of note are Hill's (1951) investigations mentioned in the anatomical part of this work. Many children in Jamaica suffer from cirrhosis of the liver as a consequence of undernourishment. A great number of biopsies, performed by Hill, confirmed Eppinger's finding that a serous exudation into Disse's spaces was always the first manifestation of the disease. It is followed by the appearance of eosinophilic coagulum in the distended Disse's spaces and around the central veins which mechanically hinders the drainage of these spaces. The next phase, according to Hill, is the oedematous dilatation of Mall's so-called periportal space, and the final stage is fibrosis. The portal lymphatics were found to be distended.

We attach particular importance to that statement of Hill that he found the Disse's spaces occluded by coagulum. It means that, *in the pathology of the Jamaican children's hepatic cirrhosis, it was the united action of the increase in sinusoid permeability, the dynamic and mechanical insufficiency of hepatic fluid circulation* (similar in its effect to our experiments where the efferent lymphatics of the liver were ligated) which produced the histopathological picture characteristic of serous hepatitis.

Our schematic drawing (Fig. 213) [illegible] *We have borrowed the first pictur* [illegible] it shows the normal structure of the l [illegible] to the liver-cell trabeculae is clearly visible; Disse's space is in reality but a virtual cleft and is illustrated as unnaturally wide in the picture in order to make it better demonstrable.

The second picture illustrates the mechanical insufficiency of the *hepatic lymph circulation. We can see the ligated lymphatics;* congestion extends from the point of ligation back to the hepatic lymph vessels, reaches the trabeculae of the liver cells and powerfully distends the Disse's spaces. Separated from the cell trabeculae, the capillaries are pushed to the centre of the Disse's spaces. The oedema of these spaces coalesces with that of Mall's periportal spaces. The third picture *depicts the liver cirrhosis of Jamaican children as described by* Hill. Disse's spaces are here quite as much distended as they appear in the hepatic lymphoedema of the second picture: we are faced here with a dynamical and not a mechanical insufficiency of hepatic lymph circulation. There appears, however, also in this case a mechanical obstruction of the intrahepatic fluid circulation: Disse's spaces contain, *here and there, eosinophilic coagula.*

It is very instructive to compare Rössle's (1944) illustration of a case of serous hepatitis caused by blackwater fever, and Hill's (1951) microphotograph of the liver of a Jamaican child suffering from hepatic cirrhosis, with the histological picture of a lymphoedema induced in the course of our animal experiments: dilatation of the Disse's spaces and *dissociation* of the liver cells are observable in the human cases

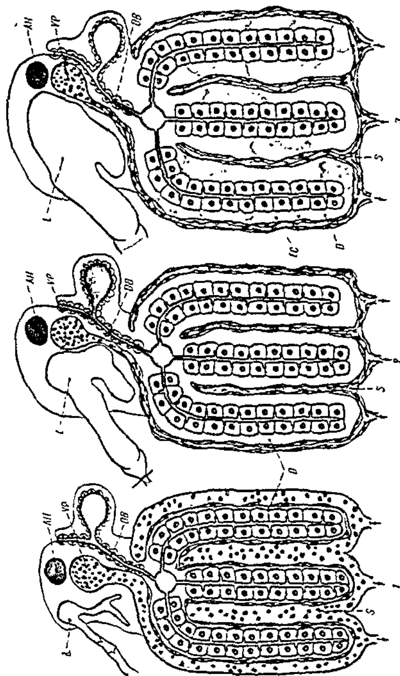



*Fig.* 213. Schema of normal hepatic structure (1), of the mechanical (2) and dynamical insufficiency (3) of the hepatic lymph flow

L = lymphatic; AH = hepatic artery; VP = portal vein, DB = biliary duct; D = Disse's space, S = sinusoid, EC = eosinophil coagulum.
For explanation of figure see text

and in the experimental hepatic lymphoedema alike (Figs. 214 and 215). Following in this respect Gloggengiesser and other authors, Popper (1951), a pupil of Eppinger, refuses to accept the term "serous inflammation" and suggests its substitution by the term "toxic oedema". We do not think that either of these expressions conform to actual facts.

Of great interest is the phenomenon that the histopathological picture of serous inflammation can be produced by a stoppage of hepatic lymph flow. Translating it into our terminology we can say *that a mechanical insufficiency of the hepatic lymph circulation gives rise to the picture of serous inflammation.* A rapid lymph flow from the

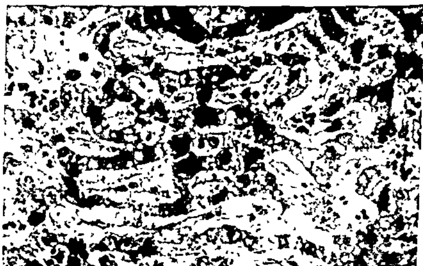


*Fig. 214.* Serous inflammation of the liver in a case of blackwater fever (Rossle 1944)

liver was observed by Eppinger in animals with serous hepatitis provoked by allyl-formiate poisoning: it is evident that, in these cases, the oedematous imbibition of the liver, the engorgement of the Disse's spaces with serous exudate, was *equivalent to the dynamic insufficiency of the hepatic lymph circulation*: the permeability of the sinusoid increased so much as to make it impossible for the lymphatics to transport the entire amount of transuded protein-rich fluid although they "worked" with full capacity. (Of course, the limited power of the liver cells and mesenchymal elements to take up proteins must also be taken into account.) Popper (1951) interprets the dilatation of the Disse's spaces in serous inflammation in essentially the same manner.

Recent literature contains a number of data, which substantiate Eppinger's results. Studying the cirrhosis of the liver induced in rats

by carbon-tetrachloride poisoning, Bollman et al. (Grindlay, Cain, Bollman and Flock 1948; Bollman 1951 a, b; Nix, Flock, Bollman 1951; Nix, Mann, Bollman, Grindlay and Flock 1951) found that the daily amount of liver lymph had *risen to many times its normal value*, while no change was observed in the amount of lymph from the bowel. We know that — according to Bollman — about 50 per cent of the total plasma of normal rats passes through the lymphatics of the liver during 24 hours; in rats affected by hepatic cirrhosis, this goes up to 200 per cent. That the permeability of the hepatic blood

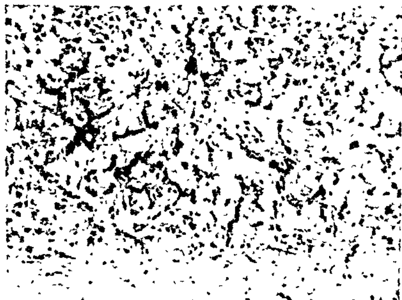


*Fig 215* Histological picture of liver cirrhosis of a Jamaican child (Hill 1951)

capillaries is increased can be inferred further from the observation that the level of protein and globulin in the lymph obtaines from the efferent hepatic lymph vessels and the Cisterna chyli of rats intoxicated by means of carbon tetrachloride is higher than the normal level, and is also borne out by the fact that liver lymph becomes bloodstained in carbon-tetrachloride poisoning.

The situation is similar in the case of the dog. Hepatic cirrhosis induced by carbon-tetrachloride intoxication, or by a constriction of the hepatic vein or the inferior Vena cava, leads to a very considerable augmentation of hepatic lymph flow: 70 to 207 per cent of the total plasma protein and 258 per cent of the total volume of circulating plasma (an increase of 500 per cent as compared with

normal conditions) pass through the lymphatic system of the liver during 24 hours.

It is thus evident that both serous inflammation and hepatic cirrhosis, its consequence, are accompanied by highly excessive lymph flow from the liver: that the lymphatic apparatus of the liver is unable to keep the Disse's spaces "dry" means a dynamic insufficiency of the hepatic lymph circulation.

We have already mentioned that, according to Kühn and Hildebrand (1951), the theory of serous inflammation cannot be applied to the liver. Their reasoning is this: if the sinusoids allow plasma [illegible] plasma proteins. Convincing as this reasoning seems to be, it leaves the quesiton open as to how one has to interpret the histopathologic picture characteristic of the liver's "serous inflammation".

We are in complete agreement with the statement that liver lymph is richer in protein than the lymph of any other organ, and the evidence of our own experimental results compels us to accept the view that a considerable amount of fluid escapes through the sinusoid walls even under normal conditions. We have seen that a lymph congestion need not last longer than some 90 minutes to cause such a lymphoedema as veritably disrupts the normal structure of the liver and thus give rise to the histological picture of serous inflammation. This, in our view, cannot mean but that *there exists, even under normal conditions, a copious flow of fluid from the blood capillaries towards the lymphatics through Disse's spaces : the rate at which fluid is produced and that at which it is carried away are, however, in equilibrium. But a displacement of this equilibrium in favour of the fluid production may suffice to bring about a situation in which the amount of protein-rich fluid pouring into the Disse's spaces per unit of time becomes higher than the transporting capacity of the efferent lymphatics* and the capacity of the liver cells to take up protein *so that the well-known histological picture of serous hepatitis is produced.* We have noted in the chapter on anatomy that the cross section of the dilated lymph capillaries is larger than that of the blood capillaries; however, lymph flow is, of course, much slower than blood flow which explains the dynamic insufficiency of the lymphatic system.

It has already been noted that the amount of lymph flowing from the liver rises to many times its original value in hepatic cirrhosis. The hepatic lymph vessels of patients suffering from cirrhosis of the liver are, as a rule, found to be excessively dilated in the course of abdominal operations (Volwiler 1951), a finding confirmed by Child (1954) who emphasizes that, periportally, no lymphatics are observable in non-cirrhotic patients. The lymphatic apparatus of the liver is insufficient nevertheless. Apart from increased requirements there is an additional factor — demonstrated by Hass (1936) in very

instructive experiments — which contributes to the insufficiency: conspicuous phenomena of irreversible regression can be observed in the lymphatics of the liver in cases of hepatic cirrhosis. "Very delicate lymph vessels — which have no direct connection on the surface of the liver with the site of injection — are frequently seen to fill with the injected substance in the incipient phase of the disease. The lymph paths traverse deeper layers of connective tissue to return to the surface at a more distant point. As the disease progresses more and more of the fine lymph vessels disappear so that at last only, a few large almost branchless lymphatics are coursing between the adenomatous nodes of the hepatic parenchyma. These channels have a comparatively large cal'bre and are usually well-filled with fluid which can be pushed to and fro so that the administration of injections becomes rather difficult."

Apart from mentioning that Hass found the capsule of cirrhotic livers cicatricially thickened and oedematously swollen, we want to quote from his work a passage concerning the significance of the constriction of the capsular lymphatic channels in the development of ascites in patients suffering from the cirrhosis of the liver:

"Irreversible constrictions of the lymphatic apparatus of the liver capsule cannot fail to affect the [illegible] the abdominal cavity. They reduce the [illegible]

It is from this surface (size, according to Wegner, 1 71 m²) that the peritoneal fluid is *secreted as transudate of the blood vessels into the clefts of the connective* tissue and thence into the open abdominal cavity, so far as it is not taken up by the lymphatics (especially by those of the liver) or reabsorbed by the blood capillaries (H. H. Meyer). The volume of the fluid which so circulates is extremely great. Isayama and Sunao, for example, estimate it at 50 times the total blood plasma per day.

Conditions of fluid exchange are quite different in cases of portal congestion. Blood pressure in the afferent vascular area rises and drainage in the efferent vessels decreases in such cases which, of course, leads to augmented transudation from the vessels to the tissues. Ascites need not, however, arise as long as the lymph vascular apparatus is unimpaired and so able to carry away the intracapsular fluid and the fluid secreted into the abdominal cavity (Quincke, Hoppe-Seyler, Umber). Congestion will not occur while the transport mechanism is intact A disturbance of transportation will occur when 1. the network of lymph capillaries are destroyed by cicatrization and larger lymphatic trunks become obstructed on the liver's surface; 2. when the entire absorptive surface becomes diminished by a shrinkage of the mesentery (Hoppe-Seyler) or the great omentum (surface, according to Wegner, 0 82 m²), the veins (Weber) and lymphatics (Maekawa) of which are obliterated; 3. when the flow of fluid between portal and efferent lymphatic system is hindered or even diverted to the abdominal cavity by cicatrized connective tissue. *All of these alterations have been demonstrated in cases of hepatic cirrhosis.*"

The findings of Hass are of general significance and of especial importance from our point of view because of his detailed treatment of the behaviour of the liver's lymph vascular system in the pathology of hepatic cirrhosis.

As we shall have occasion to discuss the origin of ascites in more detail later in this work, we do not want to dwell on this subject here and mention only in passing that, while — according to Hass — it is the reduction of fluid transport caused by a constriction of the lymphatic channels which plays a part in the genesis of ascites — several recent authors (McKee, Schilling, Tishkoff and Hyatt 1949; Volwiler, Bollman and Grindlay 1950; Davis, Lindsay and Southworth 1952; Halmágyi and Robicsek 1953) are of the opinion that, in hepatic cirrhosis, ascitic fluid and the lymph transuded into the abdominal cavity through the distended lymphatics of the liver capsule are identical.

This assumption has found full confirmation in the experiments of Mallet-Guy, Devic, Feroldi and Desjacques (1954) who pulled the liver up to the thoracic cavity and constricted the thoracic inferior Vena cava. While there resulted no accumulation of abdominal fluid in any of the experiments, a perihepatic effusion was observed in certain instances, the composition of which was the same as that of the usual ascitic fluid.

Recent data, published by Baggenstoss (1957), are worthy of note. Studying lethal cases of viral hepatitis, subchronic (subacute) atrophy and posthepatitic cirrhosis, he found that the destruction of parenchyma was followed by regenerative activity so varied in extent, duration, distribution and intensity that a wide variety of lesions resulted. Collapsed lobules, eccentrically placed vessels, and gross deformity by regenerative nodules interfere with the normal flow of blood and result in an increased flow of lymph. The number of lymphatic vessels [illegible] in the absence of further infection.

In the course of a hundred routine autopsies (without ascites) an average of 34 lymphatics was encountered in the hepatoduodenal ligament. In viral hepatitis 75 lymphatics were counted which were exceedingly distended and contained a great number of inflammatory cells. In postnecrotic cirrhosis with ascites 62 lymphatics were encountered which, too, were considerably dilated, and their walls hypertrophic.

[illegible]

## ALTERATIONS OF THE HEPATIC LYMPH VASCULAR SYSTEM RELATIVE TO AGE

It has already been pointed out that lymph flow is much more copious and the lymphatic apparatus better developed in young than in aged organisms. That this is so was observed by Mascagni

as far back as 1787 who found that the larger lymphatics of the mesentery were "dried up or obturated" in old individuals. This applies to hepatic lymph circulation as well. It was, for example, observed by Hass that the lymphatics of the liver capsule were, without injection, easily visible to the naked eye in the liver of both mature and prematurely-born infants; if an injection into the liver capsule was made, a very extensive area of profuse and delicately interwoven lymphatic vessels was seen to fill with the injected substance.

The lymphatic network of the liver becomes gradually scantier and poorer with advancing age, and it often occurs that no lymphatic network at all is demonstrable in older individuals, so that only a few isolated large lymphatic trunks, running along the capsule without lateral branches, remain observable.

## LYMPHATICS OF THE LIVER CAPSULE IN INFLAMMATION

After making injections into the lymphatics of the liver capsule of persons who had died of some infectious disease, Hass saw a network of fine lymphatics the filling of which was quite as profuse as that encountered in the liver of infants. It is highly interesting that, in such cases of inflammation, the injected substance often escapes through the wall of the lymph vessels, a phenomenon indicative of the fact that the process of inflammation is accompanied by a pathological increase in the permeability of the lymph-vessel walls. Let us refer in this connection to our experiments, discussed in an earlier part of this book, which showed that the *entry* into the lumen of the lymphatics of colloidal substances that had been injected into the interstitial space became considerably more intensive after death. This, too, points to increased permeability.

The lymphatic apparatus in the liver capsule of patients died of peritonitis, as a rule, symptoms quite similar to those seen in the body of persons who had died of some general infectious disease, and only in cases where peritonitis had lasted long does one find such an involution of the lymphatics as is usual in old age. Cases even occur where no injectable lymphatics can be found.

## SIGNIFICANCE OF HEPATIC LYMPH VESSELS IN OBSTRUCTIVE JAUNDICE

The chapter on hydronephrosis will point out the decisive part played by the lymphatics of the kidney in the pathology of hydronephrosis. Also here, in connection with the liver, are we justified in asking what will happen to the liver parenchyma after the obliteration or experimental blockage of the bile duct? Are we to accept the statement of earlier authors that increased pressure in the biliary tract caused by stasis leads to a stoppage in the production of bile, or does

the obstructed liver continue to secrete bile in the same manner as the obstructed kidney continues to secrete urine? And further, if the latter assumption is correct, does the lymphatic apparatus of the liver take part in the transportation of the bile produced after the blockage?

It is beyond doubt that the liver goes on to produce bile even after the ligation of the bile duct. This is borne out by the fact that the longer biliary drainage is prevented the more dilated the biliary tract becomes and the deeper the bile ducts "bore themselves" into the liver, parenchmya (Eppinger). The composition of the bile remains normal and its colour dark for a considerable length of time: it is only later when the hepatic parenchyma has already been impaired by the stasis, that we can see — fairly seldom — the so-called "white bile."

Fleischl (1874) was the first to demonstrate that, *after the bile ducts had been tied off, bilirubin and bile acids appeared in the lymphatics earlier than in the blood.* These experiments of Fleischl suggested the idea that it would perhaps be possible to prevent jaundice by a ligation of the lymph vessels. Investigations in this direction first seemed to confirm this assumption, but it was later established that, with an occluded bile duct, jaundice could not be prevented by the ligation of the thoracic duct. A similar conclusion was reached by Kunkel (1875, cit. Eppinger 1937).

How important a role the liver's lymphatic apparatus plays in the removal of bile in cases of obstructive jaundice was demonstrated by experiments in which the thoracic duct was cannulated and the flow of its lymph directed outward: bilirubinaemia appeared later than in cases of intact lymph flow (Gerhardt 1897; Wertheimer and Lepage 1897; Whipple and King 1911; Mayo and Greene 1929; Bloom 1923; Barron and Bumstead 1928).

Mayo and Greene (1929), as also Makino (1924), observed the appearance of bilirubin in the lymph collected from the thoracic duct not later than 15 to 60 minutes after ligation of the bile duct, whereas the bilirubin level of the blood did not rise until a few hours later. Essentially the same observation was made by Rous and McMaster (1921) and Gonzales-Oddone (1946).

Kühn (1952) examined the lymph of the efferent vessels in experimental obstruction of the bile duct. Employing the technique introduced by Cain, Grindlay, Bollman, Flock and Mann (1947), he cannulated the efferent lymphatics emerging from the lymph node of the liver, and found — 140 minutes after the obstruction of the bile duct and cystic duct — a bilirubin concentration of 8·2 mg% in the liver lymph and one of only 0·5 mg% in the blood. Together with that of the bilirubin, the concentration of bile acids and cholesterol also rose in the liver lymph. A histological analysis of the liver showed that some liver cells at the terminal ramification of the bile ducts became necrotic about 3 hours after the ligation of the bile duct.

Eppinger (1902, 1903, 1927) affirms that in cases of bil [illegible] stasis, cracks can be [illegible] open communicati [illegible] paces. The same phenomenon was described by Abranow and Samoilowicz in 1904 (cit. Barron and Bumstead 1928). That such ruptures appeared only after the congestion of bile had lasted for some time and could not be encountered at an early stage was demonstrated by the experiments of Browitz (1900), Jagic (1903), Kodama (1925) and Hiyeda (1925). Since — as has been noted — the passage of the bile into the lymphatic channels can be promptly demonstrated Ogata (1913) seems to be justified in supposing that the constituent parts of the bile can reach the lymph capillaries also by way of diffusion.

With (1947, 1949) makes the following comments in connection with these considerations:

"These observations admit of various interpretations. It is possible that delicate ruptures appear on the small bile channels through which bile is able to escape; another possibility is that bilirubin passes from the permeable bile ducts into the lymph channels by way of diffusion and filtration; a third alternative would be that — instead of the normal path of secretion, i.e. from blood to bile — there exists a differently directed secretion from the blood to the lumen of the lymph capillaries whenever pressure in the bile path exceeds a certain limit."

The possibility that the direction of bile secretion may be turned toward the lymphatic system when pressure in the bile paths becomes higher was considered by Minkowski (1892, 1904), Starling (1911), Barron and Bumstead (1928), as also by Greene (1929).

With does not think that the entry of the bile-constituents into the hepatic lymph vessels can be due to a rupture of the bile paths, seeing that it needs a comparatively long time for such ruptures to arise: Barron and Bumstead, for instance, failed to encounter them even 70 hours after the ligature of the bile duct. Increased pressure in the bile paths must — so With argues — compress the lymph capillaries which makes it impossible for the constituents of the bile to reach the lymphatic system by way of simple diffusion or filtration.

Secretion is, according to With, also proved by the fact that bilirubin is bound to protein and cannot, therefore, be filtered; against the theory of filtration argues furthermore the observation that, in cases of obstructive jaundice, the level of bilirubin is higher in the thoracic-duct lymph than in the blood.

Shafiroff, Doubilet and Rouggiero (1939) established the following correlation between pressure in the bile paths on the one hand and the bilirubin concentration in the blood and the lymph of the thoracic duct on the other hand:

TABLE 68

| Pressure in bile duct | Bilirubin content of blood | Bilirubin content of lymph |
|---|---|---|
| Below 25 cm $H_2O$ | — | — |
| Over 30 cm $H_2O$ | in 50% +<br>in 50% — | + |
| Over 40 cm $H_2O$ | + | + |

(+ = increased)

With concludes from these data that only when the pressure exceeds 25 cm $H_2O$ does the "special secretion mechanism" become operative. The experimental fact that bilirubinaemia develops also in animals with thoracic-duct fistula is explained by With on the assumption that this secretion mechanism is less "active" than that which is directed from blood to bile under normal conditions.

We feel we have to remark in connection with With's arguments that although a pathological reversal of the "directed permeability" *of the liver cells is conceivable it has never been proved, while experience* has shown that an increase in tissue tension does not as a rule lead to a compression of the lymph capillaries, for these are, *so to speak, anchored to the fibres of the connective tissue by means* of delicate fibrils so that any increase in the mutual distance of the fibrils must rather lead to a distension of the lymph capillaries. Nor does increased pressure in the bile channels result in a compression of the lymph capillaries, a fact we have had occasion to observe on our own material. What we saw in such cases was rather a dilatation of the lymph capillaries. By the assumption that an increase in the pressure within the bile paths induces a compression of the lymph capillaries, With contradicts both himself and the authors quoted by him, for — if this were so — how could the components of the bile appear in the hepatic lymph vessels earlier than in the blood?

The easiest to disprove is With's last argument. Why should the fact that, *in cases of obstructive jaundice, the concentration of* bilirubin is higher in the thoracic duct than in the blood, argue in favour of a pathological bile secretion, i.e. one directed not toward *the bile capillaries but the lymph? What in our opinion* actually happens is that, after having passed into the interstitial spaces of the liver, the bilirubin is absorbed towards both the blood capillaries and the lymph capillaries; that its concentration is higher in the lymph must be due to the comparatively more sluggish and slower flow of the lymph, while bilirubin is more diluted in the blood.

It seems that the lymphatic apparatus plays an active role also in the transport from the occluded gall bladder. *The experiments*



*Fig 216* Dilated lymphatic in the Porta hepatis. Arrows point to a valve Ligation of the Ductus choledochus. Dog



*Fig 217.* Dilated lymphatics beneath the serosa of the gall bladder after ligation of the Ductus choledochus Dog

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|---|---|---|
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carried away by the lymphatics of the liver: the survival of the liver in such cases is, therefore, *ensured by its lymphatic apparatus, just as in the same manner as the preservation of the kidney is due to its lymphatic apparatus in hydronephrosis* (see the chapter on kidney).

If the lymphatics of the liver play such a decisive role in sterile obstructions that show no infectious complication, one cannot help wondering as to the possible consequences of a ligation of the hepatic lymph nodes combined with the occlusion of the common bile duct and a simultaneous infection. What will happen if the lymphatics of the liver, an organ which has a tremendous fluid circulation and taxes its lymphatic apparatus to the utmost even under normal conditions, have to convey not merely the bile but, in addition, the products of inflammation? This question has, of course, more than mere theoretical significance seeing that bile congestion and inflammation occur, as a rule, simultaneously in human pathology.

The procedure we followed in our experiments was to expose the portal structures in the livers of cats with the technique described earlier in this book, to isolate the common bile duct and then pass a thread underneath. This done, a small incision was made on the bile duct through which we pushed a polyethylene tube right up to the liver and slightly stretched the thread. The next step was to inject 0.4 ml of coli-suspension (containing 500 million bacteria per ml) into the bile duct and to pull out the cannula while one of us tied the thread. In this manner we were able to forestall peritonitis in every case, which we tried to prevent also by a careful isolation of the operation area. The lymph nodes of the liver, also, were tied off in other experiments.

Our experiments furnished unmistakable evidence to show that closure of the bile duct combined with infection of the biliary path and a ligation of the hepatic lymph nodes, i.e. the provocation of a mechanical insufficiency of the hepatic lymph circulation results in a much graver lesion of the liver than bile congestion combined with infection but without a blockage of the lymph circulation. The dissociation of the liver cells is more pronounced and it was observed that, instead of being confined to the periportal areas, leucocytic infiltration extended intralobularly (Fig. 218).

However, we encountered also cases that were equally grave in both groups and showed no essential difference especially in regard to the extent of necrosis. Far from furnishing a proof against the decisive role of lymphatic circulation, this phenomenon should be rather regarded as its further confirmation. Marked *lymphatic thrombosis* was demonstrated in the cases of ligation of the bile duct complicated by infection in which the histological picture was as grave as that seen in cases where to infection and biliary duct-ligation an obstruction of the lymph nodes is added (Fig. 219). This would mean that a combination of bile stasis and inflammation suffices to cause an obliteration of the hepatic lymph vessels, i.e. *a mechanical insufficiency of lymph circulation* which explains the graveness of the histological picture and also explains why it is rather

of Shafiroff and Biermann (1940) with radioactive substances in connection with experimental obstruction of the cystic duct point in this direction.

These reports give rise to considerations similar to those concerning hydronephrosis: we shall see in the chapter on kidney to what consequences the obstruction of the efferent renal lymphatics leads in cases of hydronephrosis. How does the obstruction of the efferent hepatic lymph vessels affect the consequences of the ligature of the common bile duct?

In the literature there is but a single report in this connection: it was observed by Shafiroff and his collaborators (1942) that a simultaneous ligation of the thoracic duct and the bile duct led to a necrosis of the liver cells. Worthy of note as this observation is, we must be wary of drawing conclusions therefrom as bile stasis in itself may, in certain circumstances, provoke necrosis of liver cells.

The procedure we followed in our experiments (Babics, Földi, Rényi-Vámos, Romhányi, Rusznyák and Szabó 1954a, b; 1955) was that described in the chapter on anatomy: we tied off the bile duct and the hepatic lymph nodes of cats. Only the bile duct was ligated in controls a few days later. The animals were sacrificed and their livers histologically examined.

We found that the *combined ligature produced a much more serious picture than simple bile congestion.*

Previously either invisible or hardly perceptible, the efferent [illegible] distended [illegible] cir valves, [illegible] 217). [illegible] ed by our experiments, that the efferent lymph vessels of the liver become still more swollen and their contents still yellower if — after the bile duct has been ligated for a few days — we press the gall bladder or liver directly by hand. This simple observation proves in itself quite sufficiently that in obstructive jaundice there exists a practically open communication between the biliary channels and the lymphatics of the liver. It is, therefore, no wonder that a blockage of hepatic lymph circulation aggravates the consequences of simple bile stasis.

## SIGNIFICANCE OF HEPATIC LYMPH VESSELS IN OBSTRUCTIVE JAUNDICE ACCOMPANIED BY CHOLANGITIS

We have seen how great the fluid circulation of the normal liver is and how large a lymphoedema can be provoked in a short time by the blockage of lymph circulation in the intact liver. We have also noted that in cases of obstructive jaundice, when normal bile drainage becomes impossible, all the bile which is being produced has to be

difficult to make a sharp distinction between the two groups of experiments.

The occurrence of equally grave cases in both groups and a lack of essential difference especially in respect of the extent of necroses does, therefore, by no means signify that hepatic lymph circulation plays no part in the pathology of cholangitis: the point is that, in serious cases, mechanical insufficiency of the liver's lymph circulation may develop and so a serious picture arise even without a ligation of the hepatic lymph vessels.

Our results explain furthermore the observation that the clinical picture is considerably aggravated if bile congestion is associated with infection. The therapeutical conclusion from our results is that infection ought to be attacked from the very beginning, i.e. we should administer antibiotics and bile disinfectants as soon as subfebrility is found after an attack of cholelithiasis.

## THE PROBLEM OF LIVER SCLEROSIS

Rössle (1933) described — in connection with Graves' disease — the pathologico-anatomical picture of hepatic sclerosis and interpreted it as the cicatricial remainder of inflammatory processes in the substance of the liver. Rössle regards cirrhosis of the liver as the final phase in the formation of cell-rich granulation tissue, and sclerosis as a scar tissue whose fibres originate from a protein-rich exudate of the capillaries without the participation of fibroblasts. This concept of Rössle is based on the investigations of his collaborators Doljanski and Roulet (1933) who observed acellular fibre-formation in *in vitro* experiments.

We want, in connection with this theory of Rössle, to refer to our own investigations (Babics, Földi, Rényi-Vámos, Romhányi, Rusznyák and Szabó 1954a, b; 1955) concerning chronic lymphoedema of the liver (Fig. 157). Using our repeatedly-mentioned technique, we blocked the lymph circulation in the liver of cats, closed the abdomen and kept the animals alive for 2 to 3 months. Histopathological analysis revealed in some cases the presence of delicate fibres in the Disse's spaces. This observation seems to prove that the prolonged presence of protein-rich fluid in Disse's spaces may in fact lead to fibrosis and, possibly, later to sclerosis. We do not want to take sides in the controversy as to whether fibrosis develops with the participation of cells or — as is supposed by Rossle and his collaborators and also by other authors — in an acellular way.

It is instructive to compare our experiments with those investigations of Eppinger and his associates in which they poisoned their test animals by means of pyrrole and observed after some time the growth of connective-tissue fibres in the place of the protein-rich fluid that had escaped into Disse's spaces.

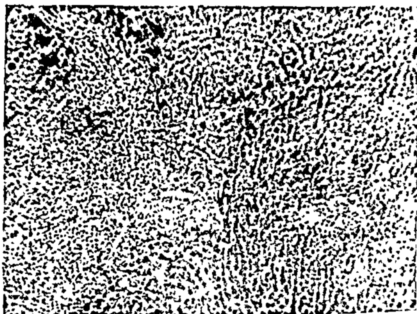


*Fig. 218* Ligation of the common bile duct + ligation of the hepatic nodes + infection. Grave oedematous loosening in the centres of the lobules. Dilatation of Disse's spaces Accumulation of leukocytes in the capillaries Intralobular extension of leukocytic infiltrations Extensive necrosis

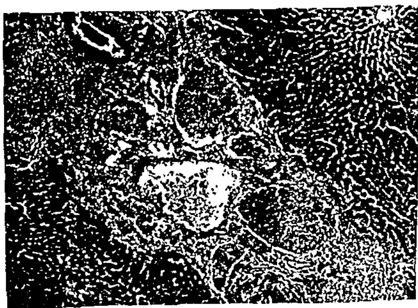


*Fig. 219.* Ligation of the common bile duct + infection. Purulent lymphatic thrombosis in periportal space

# CHAPTER XVII

# THE KIDNEYS

## PHYSIOLOGY OF RENAL LYMPH CIRCULATION

Although ever since Mascagni (1787) and Cruikshank (1790) it has been known that the kidneys contain lymph vessels, the attention of nearly all investigators has always been almost completely engaged by the vast fluid transport performed by the renal artery, renal vein and the ureter.

A great number of authors concerned themselves with the nerves of the kidneys, and many of them examined the functioning of the denervated kidney — apparently oblivious of the possibility that the efferent lymphatics may become severed during the isolation of the pedicle of the kidney in the course of denervation. How little importance authors generally attach to the lymphatic apparatus of the kidney or to its physiology and pathology is perhaps most characteristically illustrated by the fact that not more than two pages are devoted to the lymphatics of the kidney in H. W. Smith's monograph, a work that was published in 1951 and contains 1049 pages.

We find the first important data regarding renal lymph circulation in the publication of Ludwig and Savarykin (1863) who showed that the ligation of the ureter was followed by a dilatation of the efferent renal lymphatics. They failed, however, to follow up this problem. Credit for the detailed elaboration and solution of this highly significant problem is due to the scientists of the Urological Clinic of the Budapest Medical University (Babics and Rényi-Vámos 1950, 1951, 1952a, b).

Several authors have tried to determine the volume of renal lymph flow.

Schmidt and Hayman (1929), for instance, elaborated two procedures. In one of the procedures dogs were completely eviscerated and one of the kidneys was extirpated; this done, the aorta and the inferior Vena cava were tied off caudally to the remaining kidney, together with the portal vein and the hepatic artery. After cannulating the thoracic duct it was taken for granted that the fluid flowing through the cannula would represent all the lymph produced by the remaining kidney. This method was evidently unphysiological. That the injection of diuretics was followed by increased lymph flow does not allow more than qualitative conclusions.

The other method used by Schmidt and Hayman was based on the following consideration: concentration of blood has—in correspond-

## LYMPH VASCULAR SYSTEM OF THE GALL BLADDER IN INFLAMMATION

Rényi-Vámos and Jellinek (1957, 1958) observed that, after cholecystitis had been provoked by infection and a ligature of the cystic duct, the disease spread from the lumen along the connective tissue and the muscle fibres and not through the lymphatic vessels. Cellular infiltration was peri- or paralymphvascular.

Another significant observation made by them was that the experimental inflammation of the gall bladder might spread to the liver, and that the way of transmission led through the fibres of the periportal connective tissue and not the lymphatic channels. Infiltration is peri- or paralymphvascular also in this case, and the development of pancreatitis and duodenitis is of frequent occurrence.

It is evident that in human pathology we must not disregard the insufficiency of the gall bladder's lymphatic apparatus whenever we are faced with cholecystic diseases or the development of chronically atrophic cases of cholecystitis.

each kidney and 0·5 and 0·1 g, respectively, for the whole quantity of lymph produced by both kidneys every minute.

This again seems to show that the kidneys produce approximately as much lymph as urine per minute. This is a considerable amount, especially if we compare it with the amount of lymph obtainable from the thoracic duct per unit of time which, under normal conditions, is about 0·5 ml/min. in the dog. Let us refer in this connection to Fig. 168 on page 506 in which it was shown that not all the fluid introduced in the lymphatics appears in the thoracic duct because, before reaching it, part of the lymph is absorbed into the blood stream.

It should be noted that the concentration of urea in the renal lymph is considerably higher than that in the blood but lower than that in the urine. The explanation given by Sugarman and his collaborators is that renal lymph contains two components: first, capillary filtrate which has the same urea concentration as blood, second, a fluid rediffused from the collecting tubules which contains more urea than the blood. This theory has not yet been proved but appears to us more convincing than the assumption that urea is produced in the kidney.

Kaplan and co-workers (1949), after having administered inulin by the intravenous route, found that the concentration of this carbohydrate in the lymph collected from the large cervical lymph trunk amounted to 94 per cent, and in the kidney lymph only to 68 per cent, of the inulin level of the plasma. To interpret this phenomenon it is assumed by the authors that although the tissue fluid originally contains quite as much inulin as the plasma it is diluted in the kidney by the inulin-free tubular absorbate. The protein concentration of the renal lymph is, on an average, 3·79 g% according to Drinker and Yoffey (1941), and 2·8 g% according to Babics and Rényi-Vámos. Sugarman and his associates found from 0·44 to 4·21 g% (1·84 g%, on an average) of protein in the kidney lymph.

Worthy of note are the observations of Carlsten et al. (Carlsten 1950; Carlsten, Kahlson and Wicksell 1949): the lymph of the thoracic duct contains a significant amount of histaminase, about 30 times the histaminase level of the plasma. This histaminase inactivates promptly the intralymphatically administered histamine, while no histamine is encountered in the lymph after the intravenous administration of the drug. It is interesting that histaminase is transported by the thoracic duct and principally secreted by the intestinal wall. Cervical lymph and liver lymph contain no more histaminase that the plasma (Table 69).

Production of histaminase plays an undoubtedly important role in the life of the kidney; the experiments of Babics and Rényi-Vámos (1950, 1952a, b) have shown that the histamine content of the renal parenchyma becomes considerably higher in hydronephrosis, and we shall have occasion to discuss the consequences which

ence with urine and lymph production — to be higher in the renal vein than in the renal artery.

The difference can be ascertained by the haemocrit reading, so that — knowing the amount of blood flowing through the kidney per unit of time — it is possible to determine the magnitude of lymph flow. Using this method, Schmidt and Hayman found the volume of renal lymph flow to be between 0·31 and 3·22 ml per minute.

We, too, performed experiments that relied on similar considerations (Földi and Szabó 1953, unpublished). The PAH-clearance test offers a comparatively simple way to ascertaining the volume of blood that passes through the kidney. The determination of haemoglobin enabled us to find the difference between the concentration of blood in the renal vein and that in the renal artery. We employed the following formula for the determination of renal lymph flow:

$$L = C \cdot \left(1 - \frac{H_1}{H_2}\right) - W,$$

where $L$ = volume (in ml) of renal lymph produced per minute; $C$ = volume (in ml) of plasma flowing through the kidney per minute; $H_1$ and $H_2$ = haemoglobin concentration of renal artery and renal vein, respectively; $W$ = water excretion, ml per minute.

Although our results were in approximate agreement with those of Schmidt and Hayman we cannot accept this method as suitable for the determination of renal lymph flow, for in some cases, contrary to expectations, blood was found to be *more diluted* in the renal vein than in the renal artery. We are not in a position to explain this phenomenon: it can in no case be due to technical errors since Dole, Emerson, Phillips, Hamilton and Van Slyke (1945/46) who employed a quite different method (one based on the determination of the oxygen binding capacity of blood observed the same phenomenon.

Recent investigations of Pappenheimer and Kinter (1956) have proved that erythrocytes are separated from plasma in the vasculature of the kidney, a finding that may offer a possible explanation of the phenomenon in question.

It seems that — as regards order of magnitude — the figures of Schmidt and Hayman are nevertheless near the truth. This is indicated by the results obtained by Sugarman et al. (1942) who tried to determine the amount of lymph, obtainable from the kidney, by way of direct measurements. The average amount of lymph they collected from *one* efferent hilar vessel was 0·025, and the maximum amount 0·055 g per minute. If we assume for simplicity's sake that the total number of efferent lymphatics is 10 [Sisganov (1940) estimates this to be at about 12 to 16], Sugarman's figure would mean an average of 0·25 and a maximum of 0·5 g per minute for

There occurs even a functional change in the lymphoedematous kidney. When the lymph circulation of this organ was closed in our experiments it produced, on an average, 4·49 ml of urine per hour (sp. gravity, 1013) against a diuresis of 1·47 ml (sp. gravity, 1035) on the other side where the kidney had remained intact.

The concentration of chloride and urea is lower while the absolute quantity of chloride and urea excreted per unit of time is higher *in the urine excreted by the kidney with obstructed lymphatics*. The stasis of lymph causes a delay of 90 seconds in the appearance of indigo carmine, and the excretion of the dye diminish-

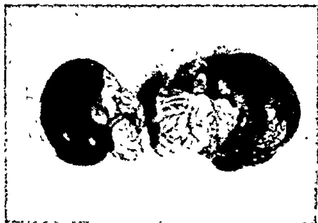


*Fig. 220.* Normal and lymphoedematous kidney The lymphoedematous kidney is swollen and larger than the normal organ

es both absolutely and relatively. Further, the urine coming from lymphoedematous kidneys contains protein and, after a few days of lymphatic congestion, histopathological alterations can be found in the tubular cells: Kaiserling speaks of "lymphogenic nephrosis".

In other experiments, Kaiserling (1940) succeeded in provoking lymphangitis by injecting pathogens into the renal lymphatics. Not insignificant was his observation that the bacteria found access to the lymph vessels of the kidney even if the efferent lymphatics were not ligated, i.e. even in the absence of a lymph congestion; of course, they migrated higher when the lymphatics were blocked. These observations are surely [illegible]

Let us add that [illegible] making the reinject[illegible] sensitization.

TABLE 69

*Histaminase contained in lymph collected from different regions*
(After Carlsten)

| Origin of lymph | Concentration of histaminase |
|---|---|
| Neck | (+) |
| Liver | (+) |
| Hind leg | — |
| Thoracic duct | ++ |
| Bowel | +++ |
| Kidney | ++++ |
| Arterial plasma | (+) |
| Venous plasma | (+) |

an increase in capillary permeability, induced by histamine, means for this organ. It is probable that the tubular reabsorption connected with the passage of various substances through the interstitial tissue leads to a continuous liberation of histamine and that renal histaminase secretion protects the kidney from the effect of toxic agents. This assumption seems to be substantiated by the fact that much histaminase is secreted also by the intestine which, like the kidney, performs an absorptive function.

Prominent among the investigations concerned with renal lymph circulation are those of Kaiserling and Soostmeyer (1939) who tied off the efferent lymphatics of the kidney in rabbits. Sugarman, *Friedman*, Barrett and Addis (1942) succeeded in demonstrating that the lymphatics became dilated after the ligation of the efferent lymph vessels, but it is Kaiserling and Soostmeyer to whom credit is due for having made a histological analysis of the kidneys and having thus examined the dilated lymphatic network of this organ right up to the lymph capillaries. This "autoinjection" of the lymphatics with lymph is, of course, a method much superior to that used by earlier authors (see in this respect the chapter on anatomy).

One of the most significant observations of Kaiserling and Soostmeyer was that the kidney was capable of swelling to twice its original volume after its lymphatics had been tied off for 10 to 15 minutes. This was due to the development of a large interstitial oedema. We made similar experiments on dogs and were repeatedly able to confirm the correctness of Kaiserling and Soostmeyer's observation (Fig. 220 shows a lymphoedematous and a normal kidney side by side).

It is now recognized by the majority of physiologists and pathologists that the tubules absorb most of the protein that is continuously escaping through the glomerular capillaries. The daily amount of protein so absorbed is surprisingly high. Supposing the protein concentration of the glomerular filtrate to be 25 to 30 mg% — a protein concentration approximately equal to that of the glomerular filtrate obtained by means of the punction of the Bowman's capsule, as also to that of the cerebrospinal fluid or the vitreous humour — and estimating the daily amount of glomerular filtrate produced by humans at 170 litres, we arrive at the result that not less than 42 to 51 g of plasma proteins are daily filtered in the kidney. It would mean proteinuria of this magnitude if the filtered protein was not absorbed by the tubules. What a protein filtration of 42 to 51 g actually means will be better appreciated if it is realized that the total volume of plasma proteins circulating in a healthy human individual of 60 kg body weight amounts to about 200 g. If the volume of glomerular filtration in the dog is taken to be 40 ml, the amount of filtere [illegible]

Terry. [illegible]
the conc [illegible]
of homologous serum, observed the appearance of proteinuria when the plasma protein concentration reached 9·6 to 10 4 g%. Their assumption that this phenomenon must have been due to the exhaustion of the capacity of the tubules to absorb protein seems to be correct: increase in the level of serum protein evidently goes hand in hand with an increase in the amount of protein filtered per unit of time which becomes so great at a given point of concentration as to exceed the absorptive capacity of the tubule cells. The situation is presumably similar to that which occurs, for instance, in glycosuria: while — under normal conditions — practically the whole of filtered glucose is absorbed, increased sugar filtration leads to glycosuria because of the inability of the tubules to absorb the excess amount of sugar. According to this theory *proteinuria arises whenever more protein is filtered per unit of time than the cells of the tubules are able to absorb.* That this is so seems to be substantiated by recent experiments of Chinard, Lauson, Eder, Greif and Hiller (1954) which they performed on patients suffering from the nephrotic syndrome. Bing (1936) set up the following formula for the determination of the *minimum* albumin concentration of the glomerular filtrate:

$$C_{alb} = U_{alb} \cdot W/C,$$

[illegible] the glomerular
[illegible] resis per minute
[illegible]

[illegible] lming majority
of nephroses, the albumin concentration of the glomerular filtrate was considerably above the normal level of 25 to 30 mg%, a finding

Romualdi and Monaci (1947a, b) repeated the experiments of Kaiserling and Soostmeyer and found their findings correct. Morphological examination revealed in the tubule cells first a "turbid intumescence" and later a picture of hyaline degeneration; fatty degeneration was not observed, nor were the glomeruli impaired.

Also cases of chronic (not more than 50-day old) lymph congestion in dogs were studied by Natucci and Zaccarini (1949) who observed the appearance of delicate connective-tissue fibres in the interstitial space and found both Bowman's capsule and the fibrous capsule to be thickened.

Why does a blockage of renal lymph circulation lead to oedema of the kidney? There exists a tremendous flow of fluid in all the other paths of renal circulation, and so the question arises: why is it that — after the obliteration of the lymph paths — the bloodstream which conveys a volume of 200 ml per minute in the dog, fails to carry off or divert towards the urine the lymph whose volume is never more than about 1 ml per minute?

In contradiction to earlier theories it is nowadays generally assumed that blood capillaries are by no means impermeable to protein: capillary filtrate always contains protein, the concentration of which varies in different organs; the removal of protein which has thus escaped into the interstitial tissue is the task of the lymphatics in the first instance, and that of the histiocytes in the second.

It is known that there are two systems of blood capillaries in the kidney: the first is that of the glomerular capillaries, while the second is composed of those capillaries which arise from the efferent arterioles of the glomeruli. Our knowledge of the composition of the post-glomerular capillary filtrate is just as scanty as that regarding the capillary filtrate of the other parts of the organism, whereas there exists ample information about the ultrafiltrate of the glomerular capillaries. As regards this primary urine, Cushny — in 1926 — still assumed it to contain no protein under normal conditions, although Morner had proved by gravimetric measurements in 1895 that there was always protein in the normal human urine (up to 155 mg per litre), a finding confirmed later by Addis (1931/32) who found the amount of [illegible] vary between *10 and 150* [illegible] *and analysing* the fluid o[illegible] [illegible]4) found that the glomerular filtrate *did not contain more than 50 mg% of protein.* Ekehorn (1931), having found protein in the glomerular filtrate of frogs and having often found the bladder-urine to be completely free from protein, came to the correct conclusion that *filtered protein was absorbed by the tubular cells.*

Walker, Bott, Oliver and MacDowell (1941) demonstrated the presence of protein in the glomerular filtrate of mammals (rats and guinea pigs).

after the intravenous or intraperitoneal injection of 150 mg/kg germanin into rats or mice, and these granules were still in the cells after the lapse of 2 months. The results of similar experiments made by Bennhold and Seybold (1932), who used proteins labelled with trypan blue, confirmed Jancsó's findings.

As regards the interpretation of these phenomena, Jancsó and Jancsó-Gábor, seeing that germanin is closely attached to the plasma proteins, refuse to believe that free germanin is continuously filtered through the glomeruli and reabsorbed by the tubules.

"We explain the histological picture by assuming that a little protein is steadily passing through the glomeruli which, as a rule, is then reabsorbed by the tubules. Should the blood plasma happen to contain germanin, it attaches itself to the proteins of the plasma so that, when the proteins pass into the epithelial cells of the tubules, they carry the adsorbed sulphonic acid with them where both protein and sulphonic acid are then stored. Thus, germanin may be regarded as an indicator which marks the physiological path of the protein in the kidney".

Does this explanation mean that, under normal conditions, proteins remain accumulated in the cells of the tubules for weeks on end? We have already expressed our view that the systematic and continuous storage of the daily-filtered amount of protein is altogether inconceivable.

Nor do Jancsó and Jancsó-Gábor suggest such possibility but offer the following highly interesting interpretation:

"Our experimental results seem to justify the conclusion that the assimilation of ingested protein becomes somehow difficult if, together with protein, molecules of adsorbed dyestuffs or germanin also gain access to the epithelial cells of the tubules. Let us bear in mind that, if protein taken up by the storage cells is destined to undergo enzymatic decomposition, one should expect germanin to hinder such decomposition since this compound is known to exert a markedly toxic effect on proteolytic processes. The experiments of Beilinsohn (1929) Town, Wills, and Wormall (1919) showed that germanin reduced the effect of trypsin to a considerable extent. Although we possess no data in respect of acid vital dyes of high molecular weight which, like germanin, become strongly attached to proteins, it is quite conceivable that a saturation of the surface of the protein particles with adsorbed dye molecules or a combination of the proteolytic enzymes with these molecules inhibits the decomposition of granules accumulated in the cells."

That germanin and the acid vital dyes behave identically as regards both their adsorption to proteins and their storage in the cells is correctly assumed by Jancsó and Jancsó-Gábor to be due to the close chemical similarity of these substances. Also afridol-violet which, like germanin, is used in trypanosomiasis was observed by them to have accumulated in the tubules.

that pointed to an increased permeability of the glomerular capillaries and an increased glomerular filtration of protein. It was further demonstrated by Lauson, Forman, McNamara, Mattar and Barnett (1954) that, when proteinuria was reduced by means of ACTH, the *effect of the hormone must have been due* to reduced glomerular filtration of protein. The possibility must nevertheless be left open that — as in renal diabetes — proteinuria may develop also in cases of normal "protein-load" as a consequence *of insufficient tubular reabsorption.*

We do not propose to enlarge here upon the question of what importance attaches to the occasionally observable histological symptoms of tubular protein absorption, i.e. whether the so-called "athrocytosis", the *epithelial storage of hyalin*, are normal or pathological phenomena, consequences of paraproteinuria as supposed by Fahr (1925) Laas (1932) Terbrüggen (1931), Randerath (1937) and Addis (1945). What, on the other hand, we must not omit to examine is the question. *what happens to the protein which passes into the glomerular filtrate under physiological conditions and is then absorbed by the cells of the tubules?*

Smetana and Johnson (1942), as also Smetana (1947), injected simple proteins (ovalbumin, serum albumin and serum globulin), labelled with the dye 2-naphthol-3:6-disulphonic acid, into test animals and found that the labelled proteins passed through the intact glomerular membrane and gained access to the cells of the tubules; the coloured droplets remained in the cells until the latter's desquamation. This observation seems to point to *storage* rather than a tubular reabsorption of the proteins.

Rather (1952), on the other hand, observed that when haemoglobin had been injected by the intraperitoneal route the absorbed haemoglobin appeared in the proximal cells of the tubules within a few hours and that, within 8 hours, the presence of free iron could be *histochemically demonstrated* in the *interior* of the cells. The haemoglobin particles disappeared promptly while the iron remained in the cells for a long time.

Rather's experiments allow of the conclusion that what actually happened in Smetana's cases was that the dye became detached from the protein and remained in the cells of the tubules, whereas the proteins escaped rapidly. We are completely convinced that protein to the extent of 42 to 51 g *per day* can in no circumstances be stored in the tubules.

The investigations of Jancsó and Jancsó-Gábor (1952a), who elaborated a technique for the histochemical demonstration of germanin (Bayer 205), deserve particular mention. Germanin is very strongly adsorbed to plasma proteins, so that the demonstration of its presence in the tissues means, at the same time, a demonstration of the whole germanin-protein complex. There appeared fine granules in the protoplasm of the tubular cells not later than 1 or 2 hours

space, expresses the view that lymphatics play no significant part in the removal of proteins because the renal parenchyma contains, according to him, only very few lymph capillaries. Eppinger attaches importance to the "interior fluid circulation" of the kidney and holds that proteins are, in the main, taken up and ingested by the cells of the connective tissue and the epithelial cells. In the course of our own experiments (Földi, Jellinek, Rusznyák, and Szabó 1954), in which the path of proteins labelled with germanin was studied, we had occasion to observe that protein which had passed into the

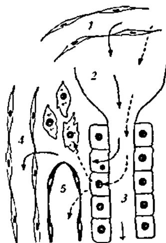



*Fig. 221.* Schema of renal fluid flow

Water and crystalloid molecules (fully-drawn arrows), and protein particles (dotted arrows) are filtered trough the glomerular capillaries *(1)* into Bowman's capsule *(2)*. All these substances are absorbed by the cells of the tubules *(3)* and forwarded to the interstitial space of the kidney. Water and crystalloid molecules are eliminated by the blood capillaries *(4)*, protein by the histiocytes and the lymph capillaries *(5)* (From Eppinger 1949, modified)

interstitial space was phagocytosed also by the cells of the renal connective tissue and by the endothelial cells of the lymph capillaries (Fig. 221).

We have emphasized our view that the cells of the tubules are unable to accumulate 42 to 51 g of protein every day; nor do we think that the interstitial histiocytes of the kidney and the endothelial cells of the vessels can digest such a tremendous amount of protein. It is undoubtedly the renal lymphatic apparatus which is the decisive factor in the removal of the proteins that have escaped into the interstitial space. It is well-known nowadays that the lymphatics of the kidney form a widespread network which is closely connected with

The experiments of Jancsó and Jancsó–Gábor, in which a protracted storage of protein labelled with germanin and acid dye of high molecular weight was observed in the tubular cells, must certainly be accepted as a proof that protein is filtered and reabsorbed but by no means prove that normal protein also may be "stored" for weeks in the cells of the tubules.

"*Tubular protein-absorption is not a passive but a dynamic process, the steady transport of protein from the tubular urine to the blood stream* which may be associated with the enzymatic decomposition of the reabsorbed protein into its simple components" — wrote Rather in 1952. Essentially, we agree with his views, provided he understands enzymatic decomposition to mean the conversion of complex proteins into simpler ones (e. g. globin may be released by haemoglobin), *but we do not think that it could mean the proteolysis* of the total amount of physiologically filtered and reabsorbed proteins.

We have expressed our opinion that a daily storage of 42 to 51 g of protein in the tubular cells seems to us inconceivable: it is a priori likewise highly improbable that 17 per cent of the organism's total plasma proteins should be decomposed in the kidney every day. Such possibility is contradicted among others by the results of recent experiments with radioactive isotopes. Vaughan, Armato, Goldthwaite, Brachman, Favour and Bayles (1952), for instance, found the half-life period of $\gamma$-globulin labelled with radioactive iodine to be 7 to 8·8 days, while Volwiler, Fremont-Smith and MacMartin (1952) found that the half-life period of the total protein marked with radioactive cystine lasted 9·6 days; that of albumin 7·1; of the total globulin 7·5; of the $\gamma$-globulin 27 days.

The experiments of Oliver (1949) furnish clear evidence to show that a connection between the structural elements of the tubular cells and the reabsorbed protein is established in the interior of these cells. Oliver succeeded in demonstrating that intraperitoneal injection of ovalbumin into rats was followed by the appearance of droplets in the tubular cells of the kidney and that these droplets were — apart from ovalbumin — composed of the cytoplasmatic elements of the cell, and contained ribonucleic acid.

We cannot agree with Rather if he interprets the expression "transport to the blood stream" to mean that protein, escaped *from* the cells of the tubules, is reabsorbed by the peritubular blood capillaries. *Why should the renal blood capillaries have the power to* absorb proteins? We are fully convinced that it is in the first place by the *lymph capillaries* that protein which has passed from the tubules to the interstitial space is taken up, together with that protein which has transuded from the nutritive postglomerular blood capillaries, and that this tremendous amount of protein is conveyed to the blood path by the lymphatic system.

Eppinger (1949), in his discussion of the serous inflammation of the kidney and of the fate of the proteins escaping into its interstitial

venously injected testicular lymph had given rise to intravascular clotting of blood in the animals.)

Natucci and Zaccarini refuse to accept the theory that one is dealing here with the storage of protein escaped through the glomeruli and reabsorbed by the tubules; they base their refusal on Randerath's statement (1937, 1948) that phenomena of this kind are induced only by foreign proteins and not by the organism's own proteins.



*Fig.* 222 Lymphoedema in the kidney. Protein-rich coagulated fluid in Bowman's capsule

Oliver (1949), in contradiction to Randerath, found that proteinuria became more pronounced *in the rat* (*rat urine* allways is known to contain some protein) and that signs of a tubular "storage" became evident when the concentration of protein in the plasma of rats had been increased by means of intravenous or intraperitoneal administration of rat plasma. Terbrüggen (1931) suggests that hyaline droplets will always appear in the tubular cells when protein, foreign to the kidney and the organism, gains access to the cells after operations, bruises or extensive haemorrhages. It is of course uncertain whether what we find in such cases is in fact paraproteinaemia and not an increased permeability of the glomerular membrane provoked by shock and serious circulatory disturbance which allows autologous

the renal secretory apparatus as also with the interstitial tissue, and we are of the view that, under normal conditions, it is chiefly due to the lymphatic apparatus that an accumulation of proteins in the renal interstices is prevented. A failure, for some reason, of the renal lymphatic system to comply with the task of protein transport leads to serious consequences: first, water is bound by the protein accumulated in the interstitial space on account of its colloid-osmotic pressure: this induces oedema in the affected organ, and we are quite in agreement with Eppinger (1949) that a process of this kind may lead to a division of the cells from the blood capillaries, i.e. to their hypoxaemia. Stagnant protein later gives rise — as is usual — to a proliferation of connective tissue, fibrosis (see the above-quoted work of Natucci and Zaccarini).

Protein is in fact continuously carried away from the interstitial space by the lymphatic apparatus of the kidney. It has already been noted that renal lymph has a very considerable protein level. If we accept 0·5 to 1 ml as the amount of lymph produced by both kidneys per unit of time, and if we base our computation on a protein concentration of 1·84 g% as suggested by Sugarman et al., we arrive at the result that, in the dog, the substance of the kidney is cleared daily of 13 to 26 g of protein by means of its lymphatic apparatus. If this calculation is based on a protein concentration of 2·84 g%, as postulated by Babics and Rényi-Vámos, or on one of 3·79 g%, as reported by Drinker and Yoffey, we arrive at still higher figures: 20 to 40 and 22 to 55 g respectively. It is worthy of note that, as regards their order of magnitude, these values are in good agreement with the amount of protein filtered and reabsorbed per day.

The importance of the renal lymphatic apparatus in the removal of proteins which are filtered and then reabsorbed under physiological circumstances is made very clear by the works of de Felice and Romualdi (1948) and Romualdi and Monaci (1947a, b) who confirmed Kaiserling's original observation that proteinuria developed in rabbits whose efferent lymphatics had been ligated. Apart from proteinuria also cylindruria was observed to have developed. They observed, moreover, a turbid intumescence in the tubular cells and, later, also hyaline degeneration. Natucci and Zaccarini (1949), too, made a similar observation on dogs: they saw symptoms of degeneration in the tubular cells "which reached the extent of albuminous swelling similar to that observable in nephrosis". We have seen that Kaiserling used the term "lymphogenic nephrosis" and interpreted these alterations — as did subsequently Natucci and Zaccarini — by the assumption that the lymph had a toxic effect and that its congestion, its retention, damaged the parenchyma of the affected organ. (Data concerning the toxicity of lymph originated from Barbéra (1898); also Rumpf is quoted by Kaiserling as having demonstrated that degeneration ensued in frog-nerves placed in frog-lymph; Kaiserling referred also to Wooldridge who had reported that intra-

lymphoedema induced by the ligature of the renal lymphatics. The tubules in Fig. 224 contain also protein casts. It is, therefore, highly probable that the histological signs of the "droplet storage" are due to proteins stagnant in and not removed from the cells; it is moreover to be supposed that there are actually toxic substances trapped in the interstitial space which have a damaging effect on the cells; it is furthermore probable that hypoxia due to the accumulation of oedema fluid, too, plays a certain part in the development of histopathological alterations.

The importance of the lymphatics in the transportation of renal protein is illustrated by the following experiment (Földi and Magasi

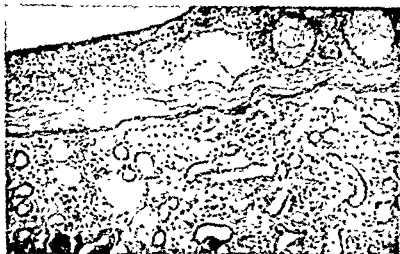


*Fig. 224.* Lymph congestion in the kidney. Interstitial oedema. *Dilated* lymphatics in capsule and cortex. Renal parenchyma perfectly intact

1954, unpublished): Using the technique described earlier in this book, we *ligated the efferent lymphatics of the left kidney in the dog.* The right kidney served as control. Bovine serum was then intravenously administered to the animal for a few days. Histological examination of the kidney revealed but feeble signs of a "storage" in the tubular cells of the right kidney, whereas the phenomenon was seen to be very pronounced in the tubular cells of the left kidney.

It will now be understood why a mechanical insufficiency of the lymph circulation is so promptly followed by the swelling and oedema of the kidney. The evidence of *our own experiments* (*Földi 1952; Földi* and Romhányi 1953a, b) enabled us to confirm the findings of Kaiserling and Soostmeyer: we found, a few hours after having tied off the *efferent renal lymphatics in dogs, grossly dilated lymph vessels and*

plasma proteins to escape through the glomerular capillaries so that the task of reabsorption becomes much heavier for the tubules. Of the Hungarian workers it was Endes (1953) who studied renal histopathology in shock, and his pictures show Bowman's capsules to contain a protein-rich fluid.

*The essence of the problem is in our opinion not the question whether autologous or foreign proteins are absorbed: the difference at issue is*

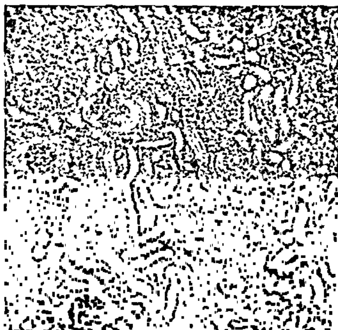


*Fig. 223.* Lymph congestion in the kidney. Protein-rich coagulated fluid in Bowman's capsule. The renal epithelial cells are not damaged

*less qualitative than quantitative; once the amount of reabsorbed protein has reached a magnitude at which its undisturbed and continuous transport from the kidney is no longer possible, the histological symptoms of tubular protein-storage will infallibly appear.* Our observation that after the ligation of the renal lymphatics the "protein accumulation" may extend from the interstitial space through the tubules back to the capsule of the glomeruli seems to confirm our concept. Fig. 222, the microphotogram of [illegible] of the thoracic duct, right [illegible] that Bowman's capsules [illegible] ated protein-rich fluid which compresses the glomeruli. Bowman's capsules, likewise filled with serum, can be seen also in Fig. 223 which illustrates a



*Fig. 226.* Lymphatic congestion in the kidney. Interstitial oedema Dilated lymphatics in capsule and cortex. Renal parenchyma not damaged

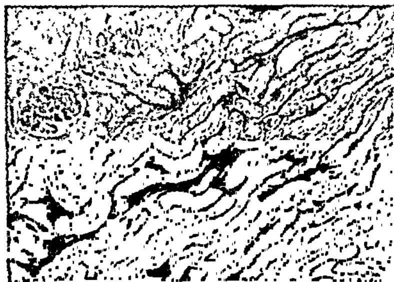


*Fig. 227.* Lymph congestion in the kidney. Interstitial oedema

interstitial oedema in the capsule as also in the parenchyma (Figs. 225, 226, 227); even the adipose tissue of the hilus became oedematous. Using the technique of fluorescence microscopy, we demonstrated a tremendous amount of proteins in the renal interstitium (Földi, Rusznyák, Szabó and Donáth 1953, unpublished): while



*Fig. 225.* Lymphatic congestion in the kidney. Interstitial oedema. Dilated lymphatics in capsule and cortex. Renal parenchyma intact

the normal control kidney, treated with Haitinger's thiazine red—thiazine yellow, showed a greenish fluorescence, the lymphoedematous kidney — obtained from the same animal — gleamed in a brownish-red colour.

It has already been pointed out that, according to Kaiserling and Soostmeyer, ligature of the renal lymphatics induces a change in the function of the kidney. We, too (Foldi 1952), undertook ex-

experimental ligation of the ureter; the only histopathological symptoms that can be observed for a considerable period are a high degree of interstitial oedema and a powerful dilatation of the lymph vessels. According to the literature, it is 3 to 4 weeks after the blockage of the renal pelvis that tubular *atrophy* becomes observable (Cohnheim 1877/1880; Lindemann 1899; Babics and Rényi-Vámos 1952). The renal parenchyma remains comparatively intact and continues to function. Babics and Rényi-Vámos (1952b) were able to demonstrate the appearance of intravenously injected inulin and indigo carmine in the renal pelvis closed by the ligation of the ureter. Jancsó (1952) succeeded in demonstrating the presence of inulin in the tubular cells of the hydronephrotic kidney. Similar results were reported by Suzuki (1912) after the introduction of carmine.

Myint (1957) claims that various substances (glucose, T-1824, inulin), injected into the renal pelvis after ligation of the ureter, are absorbed by the lymphatics of the kidney.

That the hydronephrotic kidney is able to survive and continue the performance of its functions is attributed by Babics and Rényi-Vámos to the fact that the urine which is produced passes from the renal pelvis into the interstitial space of the kidney whence it is continuously reabsorbed into the lymphatics. A ligature of the ureter does therefore, according to this theory, not produce a complete stasis of the urine since a steady flow is maintained also in this case: what happens is that the task of urine transportation devolves on the lymphatics instead of the ureter. The penetration of urine into the interstitial space leads to the liberation of histamine which, again, induces a pathological increase in capillary permeability. This, in its turn, provokes the exudation of protein-rich fluid from the capillaries. This fluid, too, is carried off by the lymphatic apparatus of the kidney. The *state of equilibrium* thus produced explains why, though obstructed, the destruction of the kidney is delayed.

If the lymphatic system of the kidney plays such an important part in the pathomechanism of hydronephrosis, the question arises: what will happen to the kidney if, *together with the ureter*, its efferent lymphatics are tied off? If the theory of Babics and Rényi-Vámos is correct and the lymphatics really play a decisive role in the pathology of hydronephrosis one is justified in expecting that lymphatic ligature cannot fail to provoke a fundamental change in the picture of hydronephrosis due to ureteral obstruction.

Before discussing the pertinent experiments (Földi and Romhányi (1953a, b), we would repeat that, *if only the lymphatics are ligated*, what follows is solely a parenchymatous degeneration in the tubular cells and never necrosis.

Dogs anaesthetized with evipan were used in the experiments. After exposing the left kidney by means of a left-side incision we tied off all efferent lymphatics, first from the direction of the posterior and then from that of the anterior hilar aspect Having ligated the lymphatics we tied off the ureter and re-

periments with a view to ascertaining the effect of acute lymphatic congestion on renal function.

The experiments were performed on dogs. After lower median laparotomy we isolated the urinary bladder and introduced polyethylene cannulae into both ureters right up to the pelvis of the kidney. We took, as a rule, several control clearance [illegible]

*experience one has no difficulty in distinguishing lymphatics from small nerves:* yet, in order to be quite sure, we made later a histological check to see that really nothing but lymphatics had been tied.

As is the case with all other lymphatic regions — so also the lymphatic apparatus of the kidney displays striking variations *in different dogs*. While the lymph vessels of the hilus and the capsule are clearly outlined in some animals without ligation, even the most careful search reveals but few lymphatics in others. As for as we could observe it is *in young animals* that the best-developed lymphatics are encountered.

The ligation of the lymph vessels is promptly followed by a swelling of the kidney, and sometimes — not always — the lymphatics of the capsule become dilated, an indication of the fact that — as has been noted in connection with the anatomy of renal lymphatics — there exists in the dog an anastomotic communication between the respective lymphatics of the kidney capsule and the parenchyma.

The lymphatics of the capsule, too, were tied off in every one of our experiments in so far as they were dilated and thus visible; in those cases in which they were indistinguishable we applied a gross ligature to the connective tissue which fixes the upper and lower poles of the kidney and in which the efferent lymph channels of the capsule run.

Having ligated the lymphatics we replaced the kidney, closed the wound and continued the experiment. The right kidney served thenceforth as control. This enabled us to make comparisons between the function of the two kidneys. The experiments were generally continued for 2 to 2½ hours after the production of lymph congestion.

We studied in these experiments the effect of acute lymph congestion on diuresis, on glomerular filtration (which was determined [illegible] clearance), on renal blood flow [illegible] maximal tubular glucose re- [illegible] p-aminohippuric-acid secretion [illegible] ium and chloride.

It was found that acute lymphatic congestion had, in general, no significant effect on glomerular filtration, renal blood flow or Tmg and $Tm_{PAH}$, and further that — *in agreement with the findings of* Kaiserling and Soostmeyer — the excretion of water, sodium and chloride became greater which increase we attributed to diminished tubular reabsorption.

## SIGNIFICANCE OF LYMPH CIRCULATION IN KIDNEYS WITH LIGATED URETER

It has long been known that the renal parenchyma shows no symptoms of grave lesion for a number of weeks after the occlusion or

capsule contained crescents of coagulated protein-rich fluid which compressed the glomerular tuft. The right kidney revealed also in these cases only those slight alterations which are characteristic of hydronephrosis.

We saw only a single case in which no necrosis had developed. These experimental results appear to us important from two points of view. First, we know of no other experiments which would have so strikingly illustrated the importance of lymph flow for the organ involved as did the necrosis of the hydronephrotic kidney with ligated lymphatics; second, our results irrefutably corroborated the correctness of the theory advanced by Babics and Rényi-Vámos regarding the pathomechanism of hydronephrosis. If the lymph-



*Fig 228* Histological picture of the kidney after ligation of ureter and efferent renal lymph vessels. Diffuse necrosis of cortex beneath the widened oedematous, slightly infiltrated capsule

atics fail to carry away the urine absorbed from the renal pelvis that has passed into the interstitial space, and also the great amount of capillary exudate formed under its toxic effect, the hydronephrotic kidney — which otherwise survives for many weeks — may be *destroyed* within a few days.

Girgensohn (1952) suggests that the thin-walled lymphatics situated between the calyxes and the vessels are compressed by the interstitial oedema arising after the occlusion of the renal pelvis so that their drainage is mechanically hindered. Translated into our terminology (Földi, Rusznyák and Szabó 1952), this would mean that, according to Girgensohn's theory, the *mechanical insufficiency* of renal lymph circulation, too, plays a part in the pathology of hydronephrosis. That a ligation of the lymphatics leads to a necrosis of the hydronephrotic kidney must be regarded as a proof of the fact that

placed the kidney. After closing the wound we made a lumbar incision on the right side, ligated the ureter of the right kidney and closed this wound.

The animals were sacrificed and their kidneys removed after 2 to 3 days. Even macroscopical inspection showed a striking difference between the two kidneys: the left was much more swollen and oedematous than the right one. The weight of the kidneys made this difference manifest (Table 70).

TABLE 70

| Experiment No. | Duration of closure in days | Weight | |
|---|---|---|---|
| | | left kidney g | right kidney g |
| 1 | 3 | 114 5 | 87·5 |
| 2 | 3 | 66·5 | 59 5 |
| 3 | 2 | 77·8 | 67·4 |
| 4 | 3 | 63 0 | 42·0 |

When the kidneys were opened it became evident that the pelvis of the left kidney was much more dilated than that of the right one.

It was, however, the histopathologic picture which revealed the *decisive difference between both kidneys. In one of the* cases, for instance, the capsule of the left kidney was found to be oedematously distended and showed sporadical haemorrhages. *The subcapsular cortical layer was completely necrotized.* There were disintegrated nuclear fragments in the interstitial space. Tubules in the deeper layers of the cortex were necrotic. Here and there in the excessively expanded interstitial tissue oedematous or haemorrhagic infiltrations were observable. Glomeruli in incompletely necrotized areas still gave a comparatively good nuclear staining. Owing to greatly increased pressure there appeared at numerous points of the Bowman-capsule half-moons consisting of invaginated convoluted tubules. A marked oedema was also encountered in the medullary substance; Henle's loops were pushed asunder, cell-nuclei were shrunken, and diffuse haemorrhages could be observed on the cortico-medullary boundary. The vessels were engorged with blood; no thrombi were seen (Figs. 228, 229, 230). The right kidney where only the ureter had been ligated showed, on the other hand, *no appreciable changes save interstitial oedema.*

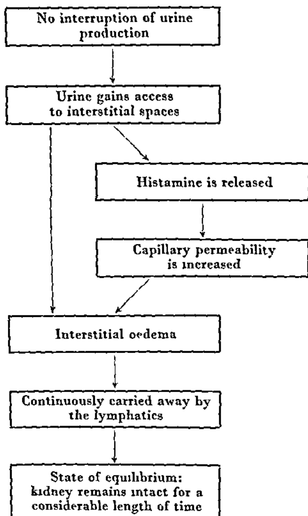
The left kidney of another animal presented a similarly grave picture, while [illegible] usly [illegible] d at [illegible] an's

the lymphatics were capable of fluid transport prior to being ligated.

atics is responsible for the development of renal oedema in hydronephrosis but their *inability to transport the increased amount of fluid.*

Schematically, the discussed processes may be illustrated as follows:

*Ligation of the ureter*



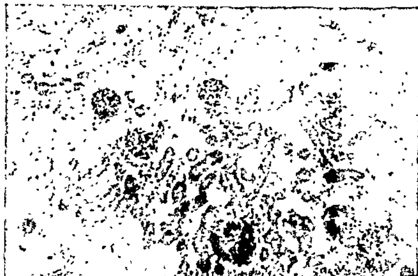


*Fig. 229.* Histological picture of the kidney after ligation of ureter and efferent renal lymph vessels. Necrotized tubules pushed apart by grave oedematous loosening of tissues. The normal structure of the kidney is disrupted. Crescent-shaped, precipitated, coagulated, protein-rich fluid is visible everywhere in Bowman's capsule



*Fig. 230.* Histological picture of the kidney after ligation of ureter and efferent renal lymph vessels. Crescent, consisting of primary convoluted canaliculi, invaginating into Bowman's capsule

to carry away as much tissue fluid as is necessary to keep the tissues "dry" and to prevent the development of oedema, it will be clear that the renal lymphatic apparatus is insufficient in hydronephrosis. Were it sufficient, it would be able to convey all urine absorbed from the renal pelvis and also the whole amount of capillary [illegible]

mulations )

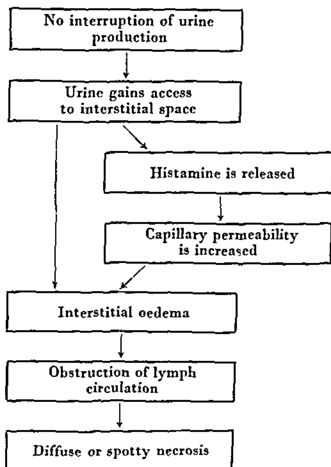
This we think explains why in hydronephrosis even incomplete lymphatic obstruction may entail serious consequences: *if, by a ligation of the essential part of the efferent lymphatics, a mechanical is added to the existing dynamical insufficiency of the lymph circulation, the lymph paths that still remain open are no longer able to ensure the pathological equilibrium in the tissues.* Toxic oedema fluid, remaining in the interstitial tissue, becomes *accumulated*, intrarenal pressure assumes extreme values and the renal parenchyma becomes necrotic.

Of significance in this connection seems to be the following statement of Babics and Rényi-Vámos (1952a, b): if, in human pathology, calculous obstruction of the ureter develops together with pedunculitis of inflammatory origin which causes a mechanical compression of the lymphatics passing through the pedicle and becomes evident during operations by the oedema of the pedicle and the swollen condition of the regional lymph nodes, it is not worth while attempting a conservative treatment, for — according to their observations — the kidney is inevitably doomed to destruction in all such cases. In cases, on the other hand, where one is dealing with a simple renal occlusion, i.e. uncomplicated by infection, Babics, led by the above-described theoretical considerations, is against the practice followed by earlier authors and favours a conservative treatment so that he is prepared to delay surgical intervention even for many weeks.

Very instructive and convincing in this respect were the experiments of Goodwin and Kaufman (1956a, b, c). They demonstrated that a ligation of the ureter was promptly followed by the augmentation of lymph flow from the cannulated thoracic duct. In other experiments, they withdrew a small quantity of urine from the closed pelvis of the kidney 24 hours after the obliteration of the ureter and introduced radioactive diodrast in its stead: the lymph collected from the thoracic duct displayed considerable radioactivity within 30 to 60 minutes, whereas no such phenomenon could be observed in the blood.

If lymph circulation plays such a significant role in sterile hydronephrosis, i.e. one not complicated by infection, the question must naturally arise: what would happen if we obstructed the renal lymphatics in cases of *pyelonephritis?* We know that inflammatory processes tend greatly to increase capillary permeability, to cause the disintegra-

*Ligation of the ureter combined with lymphatic ligature*




Here, the following question arises: why are the observed phenomena not uniformly serious; why is necrosis diffuse in one case and appearing only in spots in the next, and why did no necrosis develop in one of our cases? *Such diversity must be due to the fact that,* in all probability, one never succeeds in ligating all of the efferent renal lymphatics: sometimes one finds and ties off more of them, sometimes less. We have already noted that the lymphatics of the kidney work to "full capacity" in hydronephrosis; if interstitial oedema arises nevertheless, it must be due to a *dynamical* insufficiency of renal lymph circulation.





*Figs 231* and *232* The consequences of ureteral obstruction, if combined with infection, are considerably more serious after the ligature of the efferent renal lymphatics. Extensive, striplike infiltrations in the medulla and diffuse, patchy infiltration in the cortex are visible in the kidney after obstruction of the lymphatic vessels. (Fig 232), while only smaller striplike infiltration in the medulla and no infiltration in the cortex is visible in the kidney with unobstructed lymphatics (Fig 231)

tion of cells and the liberation of toxic substances; such inflammatory phenomena encumber the renal lymphatic apparatus at least as much as does sterile hydronephrosis. It has been proved by the experiments of Babics and Rényi-Vámos (1952a, b) that bacteria introduced into the pelvis of the kidney are carried away by the lymphatics.

It frequently occurs in pathology that ureteral occlusion is associated with *infection:* phenomena consequent upon simple renal obstruction will — according to the above considerations — occur in such cases in combination with the phenomena elicited by inflammation. Therefore, *renal lymphatics will have a much heavier* duty than in simple aseptic hydronephrosis. The situation becomes still more complicated by what Babics and Rényi-Vámos succeeded in demonstrating, viz. that the inflammation always spreads to the renal sinus and pedicle and that their adipose tissues become oedematous (peripyelitis, pedunculitis); it is therefore easily possible for the thin walls of the peduncular lymphatics to become compressed. Babics and Rényi-Vámos regard this fact as an important factor in the genesis of the pyelonephritic atrophy of the kidney. The evidence furnished *by our above-described experiments has convinced us that, in the pathology of pyelonephritis, a combined mechanical-dynamical insufficiency* of renal lymph circu[illegible] in the acute phase of the disease. Th[illegible] the well-known fact that, in d[illegible] mation in pyelonephritis soon reaches the degree of *necrosis.*

The problem we wanted to elucidate (Földi, Romhányi and Solti 1953, unpublished) was this: does a ligation of the renal lymphatics change the picture of pyelonephritis, or does a complete insufficiency *exist from the very first?*

Using the above-described method also in these experiments, we exposed the [illegible] nsion [illegible] er ml) [illegible] of the injected fluid could gain access to the abdominal cavity. [illegible] the wound, we performed the same procedure on the right side with the difference, however, that the *lymphatics were not ligated. This experimental* arrangement enabled us to study the effect of infection + hydronephrosis on the right and that of infection + hydronephrosis + lymphatic ligation on the left side of the same animal.

We made a total of 8 such experiments. Some of the animals died within three days, the rest were sacrificed on the third postoperative day. Even a macroscopical inspection revealed striking differences between the two kidneys: the kidney and its perirenal adipose tissue were more swollen and oedematous on the left than on the right side, and in some cases the left kidney *was transformed into a fluctuating purulent*

of the renal lymphatic system in cases of acute and subacute diffuse glomerulonephritis, nephrotic syndrome, amyloid nephrosis and Kimmelstiel-Wilson syndrome.

## ACUTE AND SUBACUTE GLOMERULONEPHRITIS

In cases of acute diffuse glomerulonephritis the kidney is usually enlarged, oedematously swollen, the capsule more or less stretched and can be easily stripped. If the renal parenchyma is incised the oedematous and pale cortex frequently bulges on the cut surface. All this goes to prove that the fluid content of the kidney is increased. Eppinger's investigations (1949) prove furthermore that the protein content of the kidney is exceedingly high.

TABLE 71

(According to Eppinger)

| | Dry matter % | Ash % | Water % | Protein % |
|---|---|---|---|---|
| Normal kidney | 29 6 | 1 70 | 70 4 | 14 6 |
| Glomerulonephritis | 21·4 | 1 24 | 78 6 | 26·4 |

Eppinger (1949) also demonstrated the increased protein content by histological means. He employed Haitinger's fluorochrome stains to show that the entire renal substance was infiltrated with protein in acute diffuse glomerulonephritis.

The following question was raised by Rényi-Vámos and Róna (1953): how does the renal lymphatic system behave in cases of acute diffuse glomerulonephritis? Do the lymphatics display increased activity and are they dilated in the same way as in the case of hydronephrosis when the renal parenchyma is likewise oedematous?

To elucidate this question, Rényi-Vámos and Róna examined the kidneys of 20 persons who had died of acute or subacute diffuse glomerulonephritis and came to the following conclusion:

"Interstitial oedema was encountered in every case. Mallory–Heidenhain's stain showed the oedema to be more or less developed, with the cells pale steel-grey and consequently rich in protein. In cases where death had occurred comparatively late, Mallory's stain revealed pale blue collagenous fibres in the oedematous connective tissue: these formed a profuse network in some and a less densely arranged plexus in other cases. All transitory forms between oedematous areas and hyalinized connective tissue were observable. The latter was more frequent in subacute cases where Van Gieson's stain showed it to be dense and to have a red colour.

sac, while the right kidney seemed to have well preserved its macroscopic structure. The histological picture showed that, in 7 out of 8 cases, alterations on the left side were much more serious than on the right (Figs. 231 and 232).

The conclusion is therefore justified that — as was to be expected — the renal lymphatics played a decisive part in determining the fate of the obliterated and infected kidney. Its lymphatic apparatus was insufficient in these cases and unable to clear the affected kidney of bacteria, cellular fragments and inflammatory oedema, partly because the efferent lymph ducts were strangulated by peduncular oedema which had led to a *mechanical insufficiency* of the renal lymphatic system, and partly because those efferent lymph channels which had remained unobstructed were incapable of coping with the tremendously increased work of transportation. It is thus comprehensible why a ligature of the efferent lymph vessels of the obstructed and infected kidney is followed by so serious consequences if also a *mechanical insuffiency* of the renal lymphatic apparatus is induced by the ligation of those lymph vessels which would otherwise still be able to transport fluid.

Why was it that in one of our eight cases no aggravation of parenchymal alterations could be observed after the ligation of the renal lymphatics? (The renal pelvis was more markedly dilated on the left side also in this case.) The process in this case was so grave, the inflammation and oedematous infiltration of the adipose tissue in the renal pelvis so pronounced that a practically complete mechanical insufficiency of the lymph circulation had developed in the right kidney also; therefore, an obliteration of the efferent lymphatics could no longer aggravate the histological picture. We have already noted a similar phenomenon in the chapter on hepatic lymph circulation: ligation of the common bile duct and infection of the liver with bacteria lead in certain cases to purulent thrombosis of the intrahepatic lymph vessels and so to a mechanical insufficiency of the hepatic lymph system; the histopathological picture is so grave in these cases that it cannot be further aggravated even by a ligature of the efferent lymphatics.

## SIGNIFICANCE OF THE RENAL LYMPHATIC SYSTEM IN BRIGHT'S DISEASE

It has been pointed out earlier in this work that our knowledge of the precise anatomy of the renal lymphatic apparatus is of quite recent date and that its significance for "surgical" renal diseases such as hydronephrosis, pyelonephritis, etc. has but recently been fully elucidated. We are of the opinion that the role lymph circulation plays in the various forms of Bright's disease is quite as decisive as in the so-called surgical diseases of the kidney. An attempt is made in the following to sum up our knowledge regarding the significance

endothelium of the lymph capillaries. This, also, is just a hypothesis requiring proof.

*But, whatever the reason, the consequence is certainly an insufficiency of lymph flow.*"

Being, on the whole, in agreement with the views of Rényi-Vámos and Róna we will content ourselves with just a few explanatory remarks. There can be no question of a dynamic insufficiency of lymph circulation in acute nephritis since lymph capillaries would be distended in these cases; we accept also the view that, for the same reason, a spasm of the larger efferent lymphatics cannot be considered either. We think, on the other hand, that the possibility of a spasm of the lymph capillaries should not be disregarded. We are unfortunately still ignorant in this respect and know equally little of the movements of the lymph capillaries. Worthy of note in this connection are Kaiserling's repeated observations (1940) made in the course of animal experiments: he found that even *slight stimuli closed* the smaller lymphatics and lymph capillaries *sticking their fine walls closely together:* this led to a complete obliteration of the lumen of these vessels. Eppinger's suggestion (1949) is likewise noteworthy: he thinks that the endothelial tube has an active motion of its own which enables the lumen to dilate and contract automatically and so makes it possible for the capillary walls to assume various forms.

We do not think that in acute diffuse glomerulonephritis we are confronted with that form of absorptive insufficiency in which proteins are "trapped" in the interstitial space because of their changed properties. With Haitinger's procedure it is easily demonstrable that the kidney is overflooded by plasma proteins. There exists, however, the possibility of an absorptive insufficiency of the lymph capillaries in cases of acute and subacute diffuse glomerulonephritis: it is quite possible that the hyperergia which is responsible for the capillaritis of blood capillaries and glomeruli gives rise also to lymph-capillaritis which would then disturb the normal function of the lymph capillaries.

It is worth considering the fact that the capsule of oedematous kidneys is not tight and only envelops the organ loosely in hydronephrosis, while it is as a rule taughtly stretched in acute diffuse glomerulonephritis. Does it mean that intrarenal tissue pressure is higher in acute nephritis so that is compresses the lymph capillaries?

[illegible]

to a proliferation of connective tissue, fibrosis, sclerosis and the development of chronic nephritis.

Rényi-Vámos and Róna studied the kidneys of patients who had died of acute and subacute diffuse glomerulonephritis so that it is

While more or less renal oedema was encountered in every case, only in 6 cases did we find a few lymphatics and none in the rest. Even those few lymphatics which could be seen in these cases were extremely sporadic and negligible in comparison with the number of lymph vessels usually encountered in the renal parenchyma which, in hydronephrosis, are moreover dilated."

*It thus seems evident that, in acute diffuse glomerulonephritis,* the renal lymphatic system has no share in the drainage of the protein-rich exudate that has found access to the interstitial space. Interstitial oedema constitutes *in the last analysis* some form of insufficiency of the renal lymphatic apparatus. As to the actual type of insufficiency we have to deal

*Rényi-Vámos an*

"Földi, Rusz

flow in two categories. Dynamic insufficiency occurs when the lymphatics are dilated and, though working with full capacity, nevertheless unable to render adequate assistance to the venous limb of the capillaries in carrying away the tremendous amount of interstitial fluid. Mechanical insufficiency is encountered when lymph flow is obstructed, whether on account of some organic lesion of the lymphatic channels, or because of functional reasons.

Since no, or hardly any, dilated lymphatics are encountered in glomerulonephritis, there is no question of dynamic insufficiency in these cases. It has been noted that the renal parenchyma is provided with a profuse lymphatic network which, if dilated, suffices for the transport of even considerable oedemas as it happens, for example, in hydronephrosis. On the other hand, the possibility of a mechanical insufficiency may be considered, especially one caused by a spasm of the lymphatics. This, too, is improbable since a spasm of the large lymphatic vessels ought to give rise to a dilatation of the capillaries and small vessels which, however, was not observable. There exist *still other possibilities: one would consist in some disturbance* not of

space. In such a case the lymphatic apparatus would be unimpaired and the disturbance due to a change in the proteins. This is no more than a mere supposition since not even under normal conditions do we know those factors that govern the passage of interstitial proteins into the lymph vessels and still less do we know their nature under pathological conditions. Morphology shows that the lymphatics of the renal parenchyma become dilated in certain oedemas (hydronephrosis, initial stage ( ... nephritis) and remain unchanged *in others (acute a*

Another possibility would be given

vascular walls and a consequent change in the permeability of the

the kidney. (In so doing they, of course, rupture part of the efferent lymph vessels.) The beneficial effect of decapsulation is possibly due not merely to its influence on the glomerulitis but also to its action by which the supposed spasm of the lymph capillaries is resolved.

All these are problems which we can raise but are not yet in a position to solve.

### NEPHROTIC SYNDROME

Nephrotic syndrome — called lipoid nephrosis in the terminology of earlier authors — is characterized by a considerably increased permeability of the glomerular membrane and a saturation of the tubular cells which are incapable of absorbing additional protein. A great amount of protein is daily discharged with the urine. The syndrome is, moreover, accompanied by hypercholesterolaemia and lipoiduria due to a so-far-unelucidated metabolic disturbance; the cells of the tubules are engorged with protein and lipid granules to the point of bursting.

What we want to ascertain in this connection is the role played by the renal lymphatics in the transport of proteins and lipids. It is known that in patients suffering from nephrotic oedema, oedema fluid is transported by the lymph vessels outside of the kidney, i.e. in other parts of the organism (Földi, Rusznyák and Szabó 1951c); we are confronted with a dynamic insufficiency of the lymphatic apparatus; what we want to see now is the process that occurs in the kidney itself.

It was supposed by several authors that the lipids accumulated in the interstitial space as seen in sections of nephrotic kidneys were, partly at least, situated in the lymphatics, while it was observed by Fresen (1942, 1943) that the lipid-protein crystals could be dissolved by treatment with fat solvents, and that in this case peculiar cavities filled with a protein-like substance appeared in the place of the dissolved lipid-protein crystals. Fresen claims that the cavities are arranged as a plexus which can chiefly be seen in the cortical substance around the glomeruli, along the convoluted tubules, subcapsularly, and less marked in the vicinity of Henle's loops. The cavities are [illegible]

nuclei stain dark and are frequently eccentric. Fresen found that the network was lined with preformed phagocytic endothelial cells.

The network occupies the exact place where the lymph-filled dilated lymph capillaries are visible in the experiments of Kaiserling and Soostmeyer; it is described by Fresen as not conveying the impression of a blood-filled system of tubes, so that he regards it as being composed of lymph capillaries.

It is emphasized by Fresen that the convoluted tubules contain neutral fats and other lipids and that these same substances occur also

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ning but it is likewise possible that they do not open and transport fluid until the time of convalescence. It is moreover conceivable that the lymphatics remain "passive" all along and that recovery is made *possible by a cessation of capillaritis, the "albuminuria into the tissues"*, so that the interstitial space can gradually be cleared by the lymphatics as also by the cells of the connective tissue and the endothelium.

It is known that decapsulation may entail dramatic consequences in certain rare cases of acute diffuse glomerulonephritis. Harrison, who described this operation in *1878* (cit. Korányi 1930), ascribed the efficacy of this surgical intervention to the fact that the kidney — compressed, like a glaucomatous eye, by its rigid and undilatable capsule — was freed from pressure after decapsulation. The mechanism of the favourable effect of decapsulation is not yet clear. We just want to record that decapsulation in cases of acute diffuse glomerulonephritis gives rise to the following phenomena:

1. Intrarenal pressure decreases which cannot fail to lead to improved renal lymph circulation. According to Winton (1951), intrarenal pressure drops to half of its original value after decapsulation; Eppinger observed decapsulation to have been followed by a disappearance of the cyanosis and a "reddening" of the kidney.

2. A rupture of the lymphatics occurs: therefore, if lymph flow was in fact hindered on the surface of the kidney or in the *hilum* (which, by the way, is rather unlikely), lymph, i.e. the interstitial fluid, now becomes able to pass into the perirenal tissue without hindrance. It is stated by Fuchs and Popper (1938) that, if the wound is drained after the operation, a colourless and odourless serous fluid will seep from the bed of the kidney for a number of days.

*Fuchs and Popper established the fact that, in certain* cases, the lymphatics of both kidneys empty immediately into the Cisterna chyli, i.e. without having to traverse lymph nodes, and they suggest that a kind of "correlation" between the lymph flows of the two kidneys may exist in such cases. Such a "correlation" might explain the repeatedly-observed phenomenon that a decapsulation of one kidney has a favourable effect on the other because a free *flow of the* [illegible] fluid from the decapsulated kidney facilitates lymph *flow* [illegible] is theory as rather far-fetched, for it [illegible] that lymph flow is hindered in the Cisterna chyli or somewhere above it. There is, however, no evidence to confirm such supposition since, were it true, dilated lymphatics ought to be observable in the renal parenchyma, as has been pointed out above.

3. Decapsulation means a simultaneous partial denervation. Most surgeons combine the removal of the capsule with a denervation of

kidney. (In so doing they, of course, rupture part of the efferent ph vessels.) The beneficial effect of decapsulation is possibly due merely to its influence on the glomerulitis but also to its action which the supposed spasm of the lymph capillaries is resolved. All these are problems which we can raise but are not yet in a tion to solve.

NEPHROTIC SYNDROME

Nephrotic syndrome — called lipoid nephrosis in the terminology earlier authors — is characterized by a considerably *increased* neability of the glomerular membrane and a saturation of the ılar cells which are incapable of absorbing additional protein. reat amount of protein is daily discharged with the urine. The lrome is, moreover, accompanied by hypercholesterolaemia and iduria due to a so-far-unelucidated metabolic disturbance; the ı of the tubules are engorged with protein and lipid granules to point of bursting.

What we want to ascertain in this connection is the role played the renal lymphatics in the transport of proteins and lipids. It is [illegible] ic oedema, oedema fluid [illegible] the kidney, i.e. in other [illegible] l Szabó 1951c); we are [illegible] he lymphatic apparatus; t we want to see now is the process that occurs in the kidney f.

It was supposed by several authors that the lipids accumulated he interstitial space as seen in sections of nephrotic kidneys were, ly at least, situated in the lymphatics, while it was observed by sen (1942, 1943) that the lipid-protein crystals could be dissolved treatment with fat solvents, and that in this case peculiar cavities d with a protein-like substance appeared in the place of the olved lipid-protein crystals. Fresen claims that the cavities are nged as a plexus which can chiefly be seen in the cortical substance ınd the glomeruli, along the convoluted tubules, subcapsularly, less marked in the vicinity of Henle's loops. The cavities are ounded by delicate fibres: the latter are demonstrable by tissue stains silver impregnation. Flat, endothelium-like cell-nuclei are seated the fibres. Also larger cells containing vacuoles can be seen: their lei stain dark and are frequently eccentric. Fresen found that the work was lined with preformed phagocytic endothelial cells.

The network occupies the exact place where the lymph-filled dilated ıph capillaries are visible in the experiments of Kaiserling and stmeyer; it is described by Fresen as not conveying the impression ı blood-filled system of tubes, so that he regards it as being comed of lymph capillaries.

It is emphasized by Fresen that the convoluted tubules contain ıtral fats and other lipids and that these same substances occur also

"genuine" lipoid nephrosis, and the distention of the mesenteric lymph vessels, as observed during the first operation, was presumably not due to a mechanical insufficiency of the lymph circulation but caused by that greatly increased lymph flow which is characteristic of hypoproteinaemia (see chapter on hypoproteinaemic oedema).

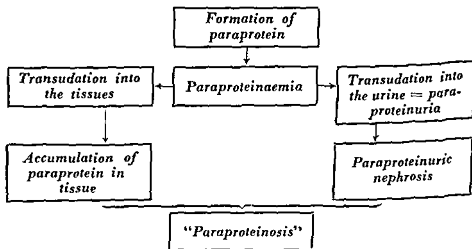
Our above arguments are, of course, not meant to convey the idea that a mechanical disturbance of the renal lymph circulation may in no circumstances play a role in human pathology: this may occur e. g. in *filariasis* or in chyluria of some other origin.

## AMYLOID NEPHROSIS

According to Randerath (1948), amyloidosis essentially consists in the following:

Amyloid is a pathological *paraprotein* which the enzymatic systems of the organism are unable to decompose and which, therefore, has to be eliminated by *transudation* ; it is — as are normal proteins — filtered in the glomeruli and then reabsorbed by the tubular cells. Processes of coacervation associated with the appearance of the paraprotein in the cells of the tubules lead to the production of hyaline drops and crystals, i.e. to *paraproteinuric nephrosis*. Paraprotein, exuded through the capillary endothelium directly into the interstitial space, is moreover deposited and accumulated in the tissues.

Randerath offers the following diagrammatic picture of the process:




Randerath claims that the amyloid deposited in the lumen of the tubules and in the tubular cells is crystalline, while that infiltrating the interstitial spaces is amorphous. This is in contradiction to the evidence of Romhányi's polarization-microscopic experiments (1949) which show that interstitial amyloid, too, has a crystalline structure.

Interesting from our point of view in these experimental results is Randerath's finding that the endothelial cells of the lymphatic capillaries are also filled with paraprotein crystals and that the lumen

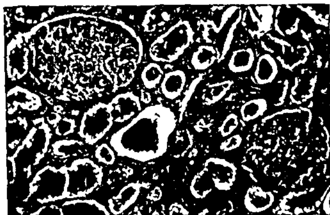


Fig. 233. Amyloid nephrosis. Protein casts in the lumen of tubules. The same substance visible also in Bowman's capsule

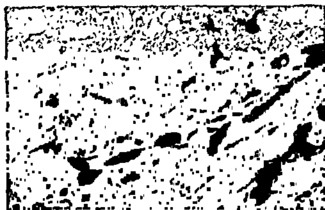


Fig. 234 Amyloid sclerosis of the kidney Condensed protein in the dilated lymphatics. Cicatrization in parenchyma

of the capillaries themselves contains homogeneous masses of paraprotein. A similar case was observed by Róna in 1953.

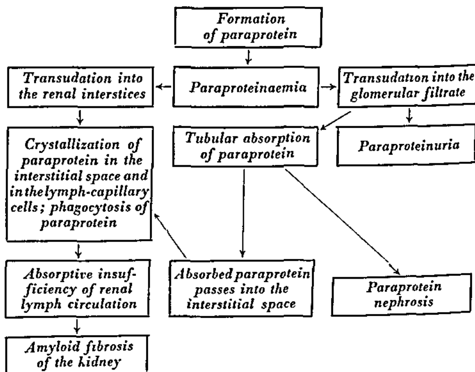
It can be seen in Figs. 233 and 234 that — condensed — paraprotein, precipitated in Bowman's capsule and in the lumen of the tubules, is encountered in the distended lymphatics also, their environment is cicatrized, a phenomenon indicative

of paraprotein having stagnated in the interstitial spaces. Paraprotein that has passed into the interstitial tissues is — for some time at least — presumably transported by the lymphatics.

It is, however, worthy of note that Randerath discovered phagocytic giant cells in the lymphatic capillaries. We regard this observation as a proof of the fact that there is no lymph flow in such cases; it is the clotted stagnant paraproteins that are phagocytosed by the giant cells.

The lymphatic apparatus of amyloid kidneys is surely insufficient. Paraproteins become arrested in the interstitial tissue, and it is — in the last analysis — due to this that paraproteinuric nephrosis develops into amyloid fibrosis of the kidney.

Led by such considerations, and taking the role played by the renal lymphatic apparatus in the pathology of the amyloid kidney also into account, we would suggest the following modification of Randerath's schema:




## LYMPHATICS AND INTERSTITIAL SPACE OF THE KIDNEY IN GLOMERULOSCLEROSIS

... concerning the clinical picture and morpho-
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(1936) it was Fahr (1912) and Randerath (1953) who described the finer histological phenomena of glomerular lesions, while Baló, Róna and Jakab (1952) contributed important data to their histogenesis. Experimental results have furnished sufficient evidence to show that it is not solely glomerular alterations with which we are confronted in cases of intercapillary glomerulosclerosis. Fahr was the first to focus attention on the hyaline thickening of Bowman's capsule and the basal membrane of the tubules. Spühler and Zollinger (1944) point

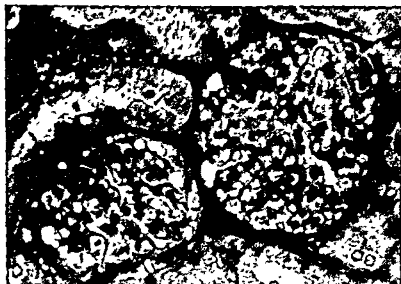


Fig 235. Intercapillary glomerulosclerosis. Proteinous imbibition of glomerular loops, Bowman's capsule and basement membrane (rabbit treated with urethan)

Randerath paid particular attention to the tubular lesions and found that they were similar to those encountered in cases of the so-called paraproteinaemic nephroses. Apart from increased permeability, he attributes a significant role to a change of plasma proteins in the pathogenesis of the disease. Increased permeability facilitates the passage of pathologic plasma proteins through the wall of the glomerular capillaries. A part of them infiltrates the basal membrane of the glomeruli and the interglomerular connective tissue, while

A survey of the literature on glomerulosclerosis makes it evident that authors did not pay much attention to lesions in the interstitial spaces of the kidney and that the renal lymphatic apparatus was practically disregarded. Pronounced sclerosis of the renal medulla is regarded by Fahr as a frequently occurring phenomenon in the glomerulosclerosis of diabetics. *It is stated by Spühler and Zollinger* that the interstitial connective tissue has proliferated in every case and contains marked cellular infiltrations. Randerath found the extent



*Fig. 236* Intercapillary glomerulosclerosis. Globules, giving fibrin reaction, visible in the enlarged glomerules (rabbit treated with urethan)

of interstitial damage varying in different cases of glomerulosclerosis. He encountered pronounced *interstitial oedema* in moderately grave and grave cases which had led to a degeneration of the connective tissue. *Serous inflammation results in a neoplasia of connective tissue* which may play a certain role in the pathogenesis of nephrosclerosis. He suggests that the glomerular loss of protein reduces the colloid-osmotic pressure in the postglomerular capillaries and that the consequential increase in filtration leads to augmented capillary transudation, in other words, to interstitial oedema. The oedematous connective tissue contains infiltrates composed of lymphocytes, plasma cells and macrophages: they are due partly to the interstitial oedema and

partly to the degeneration of the parenchyma. *A lipoid infiltration of the interstitial histiocytes is encountered in cortex and medulla alike.*

It is known that protein that has gained access to the interstitial space may induce fibrosis, sclerosis and destruction of the organ involved, a danger which the organism tries to prevent partly by means of the lymphatic system and partly with the aid of the connective-tissue cells, i.e. the histiocytes. Whenever interstitial oedema is diagnosed in any particular organ we must, therefore, raise the question as to the manner in which the lymphatics and the interstitial tissues respond to it.

The kidneys of 80 persons who had died of Diabetes mellitus were examined i[illegible] Róna, Rusznyák and Szabó [illegible] was diagno[illegible] Relying on the evidence of these 33 cases of glomerulosclerosis we investigated the histopathological picture of the kidney with a view to ascertaining the role played by the renal lymphatic system in the pathogenesis of interstitial oedema. We wanted to find out whether — as is the case in hydronephrosis — a dilatation of the renal lymphatics was demonstrable in intercapillary glomerulosclerosis or whether the picture was similar to that encountered by Rényi-Vámos and Róna (1953) in cases of acute and subacute diffuse glomerulonephritis where, as has already been noted, no dilatation of the renal lymph vessels was perceptible in spite of existing interstitial oedema.

While the analysis of our cases invariably revealed oedema or cicatrization of the interstitial connective tissue, we did not encounter distended lymphatics in any of them, and — save in a single case — lymph vessels could not be demonstrated at all. It was only in one case that we perceived partly cicatrized lymphatics whose lumen contained a protein-rich fluid (Figs. 238—240).

Seeing that it is nearly always the final stage of intercapillary glomerulosclerosis which one encounters at autopsy and that, consequently, such material cannot give us information about its initial phenomena, we were compelled to investigate cases of experimental glomerulosclerosis in order to be able to observe the behaviour of the lymph capillaries at the outset of the disease.

Juhász, Baló and Kendrey (1953) induced experimental glomerulosclerosis by administering urethan to mice, while Róna (1953) succeeded in producing typical glomerulosclerosis in 8 out of a total of 30 rabbits; glomerulosclerosis or a cicatrization of the glomeruli was observable in every case. It is characteristic that the postglomerular capillaries were maximally distended in nearly every case and that cylinders of paraprotein formed in the tubules. Together with Róna, we re-examined the said material in respect of the lymph vascular system and were able to observe dilated renal lymphatics in two cases only. In one of them, glomerulosclerosis and a large renal oedema was observable on the 16th experimental day already, while

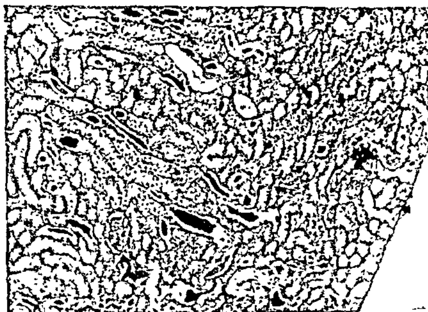


*Fig* 237. Intercapillary glomerulosclerosis. Interstitial oedema in rabbit treated with urethan (Mallory's stain)



*Fig* 238. Intercapi... ph vessel in the medulla of the ... the glomerular loops Intersti... ears, glome-rulosclerosis, Mallory's stain)



*Fig* 239 Intercapillary glomerulosclerosis Interstitial oedema. Grossly dilated lymphatics (rabbit treated with urethan)



*Fig* 240. Intercapillary glomerulosclerosis. Cicatrization in the interstitial space. Disintegration of tubules, dilated lymph vessel (rabbit treated with urethan)

in the other case not merely interstitial oedema and sclerosis but also a cicatrization of the glomeruli was visible. A single lymph vessel was seen in each of 4 additional cases. Although a more or less advanced oedema existed in 8 of the remaining 24 cases, *in none of them did* we encounter lymph vessels.

Our investigations thus furnished evidence to show that — in contradistinction to hydronephrosis — the renal lymphatics reveal no signs of increased fluid transport in cases of intercapillary glomerulosclerosis; the lymphatic vessels of the kidney remain passive and their behaviour is similar to that in acute and subacute glomerulonephritis.

Why does the lymphatic system behave in this manner? Of what type is the insufficiency of the renal lymphatics?

The case at issue is surely not one of dynamic insufficiency where the renal lymphatics work "at full steam" but, on account of their limited transport capacity, are unable to prevent the organ from becoming oedematous because in such case the lymphatics ought to be distended. Neither is one dealing with some form of mechanical insufficiency where the mechanical obstruction of lymph flow lies somewhere outside of the organ because the lymphatics of the paren- [illegible] however, [illegible] lymphatics we are inclined to assuming the existence of a special type of mechanical insufficiency which would be due to an intralymphatic c[illegible] the lymph vessels and [illegible] vessels must be dc[illegible] able whether such lymphatics embedded in the surrounding cicatricial tissue can always be recognized.

One might think also of an absorptive insufficiency of the renal lymphatic apparatus; the protein that has passed into the interstitial tissue may be a paraprotein "arrested" in the interstitial space; it is also possible that the normal functioning of the lymphatic capillaries becomes disturbed, nor is it impossible that one is confronted with a change in the function of the endothelial cells induced by the intracapillary accumulation of paraprotein.

## ROLE OF LYMPHATICS IN THE ORIGIN *OF RENAL CALCULI*

It is not proposed to expatiate upon the various theories concerning the origin of renal calculi and we will content ourselves with a reference to the latest investigations which point to the fact that the lymph vessels of the kidney play a certain role in the genesis of renal calculi.

It was demonstrated by Anderson and McDonald in 1946 that, under normal conditions, tiny figures of microscopic dimensions — so-called microliths — are situated in the renal parenchyma. Carr (1954) suggests that these microliths are continuously removed by the renal lymphatics from the interstitial tissue in the same way as particles of lampblack that have gained access to the pulmonary parenchyma of the lungs are carried off by the pulmonary lymphatics. If — for some reason — the microliths become "trapped" in a lymphatic they will grow first intravascularly but will later break through the lymphatic wall (it is as if they induced decubitus), gain access to the calyxes and develop into renal calculi. Carr mentions two factors as being responsible for the failure of lymphatic transport.

1. The microliths grow to excessive dimensions (hyperparathyroidism; disturbed calcium metabolism; change in protective colloids, etc.).

2. Lymphatic obliteration; great significance attaches in this respect to infections which may lead to lymphangitis, perilymphangitis or a closure of lymph vessels.

Carr bases this theory on various observations. Using the microradiographic method, he found, for instance, even macroscopically visible formations in completely normal kidneys that were larger than microliths: he demonstrated one or two such concretions in the kidney of all persons above the age of 9 years. These concrements are cylindrical and lie in smooth-walled cavities regarded by Carr as enlarged lymphatics.

Other experiments performed by him with the aid of diffraction X-ray analysis showed that, in cases of nephrolithiasis, these concretions had exactly the same structure as renal calculi. Histological investigations revealed that the concretions observed in the renal parenchyma were frequently situated in endothel-lined lumina. Such intraluminar concrements were always seen in areas with a rich lymphatic supply: at the cortico-medullary boundary, subcapsularly and along the interlobular vessels or outside the fornix calycis. Concrements were encountered also in the peripelvic adipose tissue.

Worthy of note is the observation of Carr that many more microliths and concrements are observable in the parenchyma of the nephrolithic renal segment than in normal kidneys. This seems to confirm the assumption that a connection exists between the formation of microliths and concrements on the one side and nephrolithiasis on the other.

## CHYLURIA

Urological literature uses this term to denote that infrequent phenomenon which consists in the appearance of chyle (fat!) in the urine. Chyluria is sometimes accompanied by haematuria. Two pathological changes are necessary for the development of chyluria: 1. increased pressure in the area of the lymphatic system of the abdominal cavity

# CHAPTER XIX

# THE PANCREAS

Our experimental results, described in the foregoing chapters, whi [illegible] atic [illegible] the [illegible] fect [illegible] the [illegible] by [illegible]

Papp and his co-workers proceeded in the following manner:

About 5 hours before the operation, the animals ingested some 2 to 3 dl of table oil. The closure of the pancreatic duct was performed in anaesthetized animals under aseptic conditions. The duodenum was opened at its free end, and the ampulla of Vater closed by a few stitches from the direction of the lumen, together with the orifice of Santorini's duct at a distance of about 3 cm distally from the ampulla. It was thus possible to avoid the injury of the pancreatic parenchyma during operation, a necessary precaution because any injury of the parenchyma is in itself sufficient to make the pancreas necrotic, especially if the abnormal course of an efferent tube necessitates a major operation.

The bile duct was also tied off. This had the object of preventing the flow of bile into the pancreas and, on the other hand, to relieve the sutures from the great pressure of the stagnant bile. (The common bile duct was tied off in the controls also.)

The intestines were regularly closed, care being taken that no passage disturbances should arise.

Congestion of lymph was induced by the ligation of the thoracic duct in the neck.

The animals were killed by an overdosage of the anaesthetic 12—24—48 hours after the operation. Benda's reaction was employed to demonstrate fat necrosis: areas with fat necrosis stain green if stained with diluted copper acetate.

Papp and his collaborators found a fairly widespread fat necrosis not later than 17 hours after the ligation of the pancreatic efferent duct and the thoracic duct: this necrosis had grown to serious dimensions 24 and 48 hours after the operation. The necrotic foci were situated intrapancreatically, but especially extrapancreatically. They were nearly always seen in the proximity of the lymph nodes on the root of the mesentery, on the mesentery itself, in the preperitoneal adipose tissue and also perirenally. In one case, the fat necrosis extended to the pericardium and the wall of the thorax.

A correlation between lymph congestion and the size of the necrotized area was observable: the graver the chylous lymph congestion, the more widespread the fat necrosis. The direction in which fat necro-



*Fig. 241.* Diffuse necrosis of the pancreas caused by ligation of its ducts combined with lymphatic stasis

sis was spreading seemed to coincide with that of retrograde lymph congestion.

In the control animals — in which only the pancreatic duct and the bile duct had been ligated, no necrosis was visible after 12 hours; even after 24 and 48 hours only millet- or lentil-sized necrotic foci became macroscopically observable.

How can these interesting experimental results be applied to human pathology? It is known that pancreatic fat necrosis in man occurs more frequently in cases of advanced cholecystitis and choleli-



*Fig. 242.* Diffuse necrosis of the pancreas caused by ligation of its ducts combined with lymphatic stasis

thiasis. These recurring inflammatory processes may spread to the lymphatics surrounding the pancreas which drain also the efferent pancreatic lymphatics; lymphadenitis, lymphangitis, lymphatic thromboses may arise leading to a reduction of the transport capacity of the efferent lymph channels of the pancreas.

It is known moreover that abundant meals also play a role in the pathogenesis. Of course, abundant meals mean a great strain for the

whole abdominal lymph circulation; if part of the lymphatics is occluded because of advanced inflammation, a dynamic insufficiency of the pancreatic lymphatics may easily arise. There occurs, at the same time, a more vigorous secretion of the digestive enzymes in the pancreas. It may happen that the efferent pancreatic duct becomes obstructed functionally by spasm and organically by calculi, metaplasia, sometimes even by worms.

In pancreatic fat necrosis, procaine block frequently gives good results. Papp and his co-workers are right in assuming that lymphangiospasmolysis might be induced by this intervention and that, thus, the transportation of enzymes through the lymph paths might be improved.

In connection with these interesting experiments, let us also mention that of Popper and Necheles (1940): they demonstrated that, after the ligation of the pancreatic duct, pancreatic enzymes were more abundantly drained through the thoracic duct. They also found that the administration of secretin raised the diastase level of the thoracic-duct lymph.

## CHAPTER XX

# THE THYROID GLAND

The chapter on the anatomy of the thyroid lymphatic system contains the description of experiments in which we induced a mechanical insufficiency of this system in dogs by the ligature of both cervical lymph trunks.

The present chapter discusses the insufficiency of the lymphatic [illegible]

*that of the acini* (Figs. 243—248). *Beside these dilated lymphatics in the substance of the thyroid gland an extralymphvascular fluid could also be seen which had the same appearance and stained in the same manner as the content of the lymphatics :* traces of the original undulated collagenous fibres were clearly visible in this fluid. *The wall of the dilated lymphatics was ruptured* at certain points, evidently as a consequence of increased tension; it is there that lymph extravasates into the tissue between the acini.

The free "lymphoedema" pushes the acini apart.

The oedema fluid pouring forth from the bursting lymphatics which pushes the acini asunder is similar to the intralymphvascular fluid. In the same way as the lymph which gives a multi-coloured staining between the acini that contain multi-coloured colloids, the extravasated oedema fluid between the acini gives a multi-coloured staining. If we follow the destiny of the lymph contained in the dilated lymphatics and that of the extralymphvascular "lymphoedema fluid" we find the following: *fibres begin to be formed at certain points in the dilated lymphatics* which may be observed with Van Gieson's stain. Precisely the same phenomenon is observable in the oedema fluid which presses the acini apart. There are often no cellular elements visible in the region of this fibrogenesis, so that one is possibly dealing here with *acellular* fibrosis. [illegible]

first pink and then red. Mallory's stain enables us to see the [illegible] development of *blue-staining hyaline fibres.*

It can easily be perceived that these phenomena develop by degrees in the parenchyma of the thyroid. *While only a congestion of lymph is observable at first, the advancement of the lymphoedema induces a*

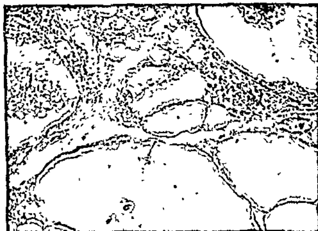


*Fig. 243.* A large lymph pool arises from the lymphatics. Strongly dilated acini (Man. Van Gieson's stain)



*Fig 244* Extended oedema between the acini. Dilated, large lymphatics Fibrogenesis visible in the oedematous fluid (Man Van Gieson's stain)

*gradually increasing disintegration of the thyroid's normal structure. Thereafter, the formation of fibres in the oedema fluid begins* which —

*remains normal.*

It is important to point to the fact that *the endothelial cells of the lymphatics in the diseased regions are often strikingly swollen and inflated.*

The lymph vessels in the intact or comparatively intact parenchyma remain normal.

*In more advanced stages* lymphatics running between the hyalinized areas also become hyaline and obliterated.

As has been mentioned, we observed the development of lymph congestion in cases where the substance of the thyroid gland contained adenomas of a small or medium size. Oedema in such cases was largest in the area of adenomas surrounded by a capsule of connective tissue; we saw, at the same time, a considerable congestion of lymph in the

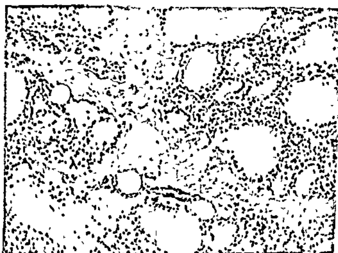


*Fig. 245.* A dilated lymphatic is visible, the contents of which pass into the surrounding oedema. Oedema fluid pushes acini apart. Extended formation of fibres in the oedematous fluid (Van Gieson's stain)

substance of the thyroid around the adenoma. In more distant areas neither lymph congestion nor oedema were found.

In certain cases *we also saw a slight leukocytic infiltration in the thyroid parenchyma, simultaneously with lymph congestion.*

We also investigated 27 cases of *colloid* goitre. We found *in this* material *the same histological changes in a diffuse and extended form as had been observed in a focal arrangement in the macroscopically normal thyroid of adults.* This observation gives rise to an important and intriguing question: is the difference between the phenomena observed in normal thyroid glands and the colloid goitre an essential, i. e. qualitative, or merely quantitative one? Our experimental results incline us to regard the latter alternative as probable. We cannot, however, decide this question until a much larger series of material has been analysed.



*Fig.* 246. Azan stains oedema fluid red. Extended hyalinization of oedema fluid



*Fig.* 247. Disintegrating acini, inflammatory phenomena. Formation of fibres in a disrupted lymphatic (Van Gieson's stain)

We also investigated 20 cases of *Graves' disease*. We encountered lymph vessels in these goitres, but they were scarcely dilated and none of them displayed pathological symptoms.

A special series of experiments was devoted to *experimental chronic lymph congestion*. In dogs we tied off both cervical trunks and found, 8 weeks later, the development of *intralymphvascular* fibrosis in the dilated thyroid lymphatics (Fig. 249). We thus succeeded

The lymph vessels in the intact or comparatively intact parenchyma remain normal.

In more advanced stages lymphatics running between the hyalinized areas also become hyaline and obliterated.

As has been mentioned, we observed the development of lymph congestion in cases where the *substance of the thyroid gland contained* adenomas of a small or medium size. Oedema in such cases was largest in the area of adenomas surrounded by a capsule of connective tissue; we saw, at the same time, a considerable congestion of lymph in the

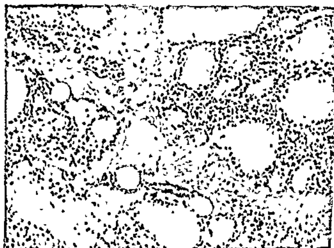


*Fig. 245.* A dilated lymphatic is visible, the contents of which pass into the surrounding oedema. Oedema fluid pushes acini apart. Extended formation of fibres in the oedematous fluid (Van Gieson's stain)

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which, however, he says himself that "this attempt of an explanation has but little probability". The point is now, *to which type* the insufficiency of the lymph flow in the thyroid gland belongs. The following alternatives are possible:

a) The insufficiency of the thyroid lymph flow may be a *mechanical* one. We think the first pathological alteration is the appearance of a small adenomatous node. The adenoma is enclosed in a small capsule of connective tissue which compresses the surrounding lymphatics. This may lead — as has been pointed out — to the oedema of the adenoma and its direct surroundings.

b) We have mentioned that the endothelial cells of the lymph capillaries are conspicuously swollen and inflated in the diseased area. This morphological feature allows the assumption that these cells do not function normally: there arises, therefore, the possibility of *an absorptive insufficiency* of the thyroid lymphatic system.

c) It has been likewise noted that sporadic inflammatory infiltration is demonstrable. The question of priority is not yet settled: we do not know whether it is the inflammation which leads to exudation, increased lymph flow and a dilatation of the lymphatics, or whether inflammation becomes secondarily associated with the already existing lymphoedema. If the inflammation is a primary phenomenon, we are dealing with the *dynamic form* of lymphatic insufficiency.

d) We must finally assume that either the colloid of the thyroid gland or the composition of the interstitial proteins is so changed as to give rise to that form of *absorptive* insufficiency which causes proteins to become "trapped" in the interstitial space.

We consider it most likely that these different phenomena occur simultaneously and mutually aggravate one another: the connective-tissue capsule of the adenomatous node hinders lymph flow mechanically; the endothelial cells of the lymph capillaries become diseased at the same time; the composition of the colloid and the interstitial proteins is changed; inflammatory changes are produced; intralymphvascular and extralymphvascular cicatrization begins, etc.



*Fig. 248.* Extended hyalinization. Only a few acini and a small lymphatic recognizable (H-E stain)



*Fig. 249.* Signs of fibrogenesis in a dilated lymphatic with acini pushed apart, 8 weeks after the ligation of both cervical lymphatic trunks (Van Gieson's stain)

*in reproducing phenomena, observed in human pathology, also in animal experiments.*

Relying on the evidence of our experiments we think we are justified in claiming that *a certain kind of insufficiency of the thyroid lymphatic apparatus is involved both in the pathological phenomena arising in advanced age and in the development of colloid struma.* Our theory is, therefore, in agreement with the concept of Müller (1896) regarding

From our point of view, Jäger's concept is particularly interesting and seems to be the best founded of all theories:

"The reconstruction made of serial sections revealed vascular plexuses in the connective-tissue coat at the ramifications of the arteries: these plexuses united to form one or more parallel trunks. They ran distally as far as the next follicles, and proximally along the arterial adventitia, without showing, however, arterial or any other kind of vascular origin. Such a trunk was followed in serial sections up to the point where the trabecular artery and vein separate. The irregular sinus-like dilatations and the peculiar islets (ramification and reunion) gave the impression of lymph vessels, and a comparison with the course of the above-mentioned carcinomatously infiltrated lymphatics proved the correctness of this conclusion. In cases of peripheral congestion — irrespective of whether it is continuous, produced by the occlusion of splenic vein, or recurrent as a result of the hindrance of portal circulation — an extraordinarily increased amount of lymph is flowing through these lymph paths towards the hilus. The tissue fluid stream-

therefore remain and age on the spot, undergo there gradual disintegration and enrich the lymph with the waste products of blood. So it happens that the arterial walls become saturated with iron salts although the pulp of the spleen is usually not rich in iron. These iron salts are harmful for the structure of splenic tissues. The collagenous and especially the elastic fibres of the arterial wall become diffusely saturated with iron, often only on that side of the artery where the lymph paths are running".

Things are the same if the splenic lymph flow is not increased but the disintegration of erythrocytes becomes intensified (haemolytic icterus, drepanocytic anaemia, splenic infarcts).

Deposits of haemosiderin can be demonstrated as far as the regional lymph nodes

Jäger substantiated his theory by means of animal experiments: by tying off the lienal or the portal vein of dogs, he produced a picture which was similar to that observable in human subjects.

The Gandy–Gamna nodes themselves are, according to Jäger, infarcted Malpighian bodies, the blood and lymph circulation of which is impaired by advanced fibrosis.

Encouraged by Jäger's work, Natucci and Giarelli (1951) also performed experiments on animals. They ligated the efferent lymph trunks running in the splenic hilus of the dog. The animals were then killed at different dates, from 2 days to 2 months after the operation. They found the following:

The colour of the spleen had remained unchanged but its consistency became somewhat more compact after a lymph congestion of 2 to 7 days.

# CHAPTER XXI

# THE SPLEEN

Jager (1937a, b) was the first to demonstrate that a pathological change in the lymphatic system of the spleen plays a decisive role in the origin of certain splenopathies.

In connection with Banti's disease, the origin of periarterial fibrosis is one of the most controversial questions. In periarterial fibrosis, the follicular — and frequently also the prefollicular — portion of the splenic arteries is surrounded by a zone of thick connective tissue. According to Dürr (1924), fibrosis extends to the whole length of the arteries; we encounter the same phenomenon also in cirrhosis of the liver. This was later confirmed by Jäger. He observed periarterial fibrosis of a high deg[illegible]
in 15 cases of hepatic [illegible]
cases of a simultane[illegible]
Jäger suggests that periarterial fibrosis is a consequence of chronic productive lymphangitis and perilymphangitis. These arise in the following way: phlebohypertension, a great amount of lymph — loaded with metabolites and blood — flows through the lymphatics [illegible]

sharply circumscribed, hard, tobacco-coloured, millet-sized structures, seated at the ramification of arteries, to aspergillus infection. Others think of a dystrophic origin: "The ferruginous, partly segmented structures inside the nodules are encrusted, destroyed fibres; the spherical and pear-shaped ones are colloidal precipitations of ferrous compounds. However, the question remains unsolved (De Vecchi): What is it that leads to the focal damage of the connective tissue, and to the precipitation of iron salts in the spleen the pulp of which is often far from being rich in iron?" — writes Jager.

We must remark that the Gandy–Gamna nodules always arise in cases of lienal phlebohypertension, in cirrhosis of the liver, pylothrombosis, thrombosis of the lienal vein or its compression by tumours or a dislocation of the spleen, i.e. when the periarterial fibrosis referred to above is present. The Gandy–Gamna nodes are always seated on arteries and never on veins. Jäger found this confirmed by the observation that the nodes gave X-ray shadows when roentgenograms were made of the splenic veins that had previously been injected with air.

Eppinger (1920) and Abrikosov (1929) attribute the Gandy–Gamna nodes to the rhexis and Dürr (1924) to an injury of the arterial wall.

after the operation showed marked histological changes: considerable congestion of the central veins and the sinuses, oedema of the porto-biliary area and swelling of the Kupffer's cells; the hepatic cells themselves were still not damaged at this stage. Congestion in the liver of animals that were sacrificed 3 to 4 months after the operation was, however, of such a high degree as to have flattened the hepatic cells at several points; cell boundaries were effaced, the cytoplasm had become granular and showed sporadic biliary pigmentation. The expansion of the sinuses was marked, and frequently also the Disse's spaces were dilated. Endothelial cells were swollen. Around the blood vessels cuff-like proliferation of connective tissue was found. Also the capsule [illegible] they poi[illegible] lymph circulation in the spleen. It has already been mentioned that the lymph circulation of the spleen participates in the maintenance of splenic fluid circulation in cases of portal and lienal phlebohypertonia (appearance of haemolymph nodes); the work of Natucci and Giarelli has made it clear that the occlusion of lymph circulation in the spleen pr[illegible] w[illegible] pi[illegible] it[illegible] atics of the spleen, histopathological changes arise in the liver also

Whitish threads showed the course of the lymphatics on the lower surface of the spleen. The sections revealed intensive congestion and slight hyperplasia of the lymphatic apparatus.

After a lymph congestion of a month, the spleen was much more rigid than normally; the surface of the organ was granular and the capsule obscured by whitish spots in its portions closest to hilus; the blood capillaries were dilated and the lymphatics running along them showed as whitish bundles. Follicles of various sizes were visible on the surface of the sections. Some of them were almost invisible to the naked eye, while others had a considerable size. The parenchyma was covered with these tiny white points to such an extent that the picture became similar to that of tuberculous granulation. The capsule was thickened and even the liver was swollen.

In the animals in which the congestion of lymph had lasted 3 to 4 months a considerable increase of the spleen's consistency and a slight augmentation of its volume were observed. The shape of the spleen, too, had changed: its edges were extended and had grown coarse and knotty. The follicles appeared as tiny dots on the surface of the sections. Of the other organs it was only the liver which showed signs of a change: it seemed to be considerably hyperaemic. Moreover, numerous red haemolymph nodes were visible on the mesentery.

Very interesting was the result of the histological examination. In all cases where lymph congestion had lasted only a few days, a striking dilatation of the lymph capillaries could be perceived, so that not only the lymphatics running along the larger blood vessels but also those of the red pulp were dilated. After the 7th day, the dilatation of the lymph capillaries became very marked. The cellular elements of the pulp became scarcer. The capsule was somewhat thickened and the germinative centres of the Malpighian bodies markedly hyperplastic.

After a month of lymph congestion, the most conspicuous phenomenon — apart from the thickening of the capsule — was that the cellular elements of the pulp had become scantier and that follicular hyperplasia had also developed.

A pronounced damage of the entire splenic parenchyma could be observed after the lapse of 3 to 4 months. Worthy of note is also the observation that the periadventitial collagenous cover of the blood vessels was thickened. Both the smaller and the larger blood vessels ... proliferated
ntral
sule
s: it
or in
cells and showing no inflammatory phenomena.

No significant histological lesions could be observed in the liver of animals in which the hepatic lymph congestion had lasted 2 to 7 days, while the liver of the animals which were examined a month

Experimental evidence for the theory that a lasting obliteration of the lymphatics is the pathological change common to all these cases has been provided by the fundamental experiments of Drinker, Field and Homans (1931) which have repeatedly been referred to in this monograph. A simple ligation of the lymphatics of a limb in the dog produces no consequences. It is due to this fact that certain authors have reached the conclusion that the obliteration of the lymphatics does not, *in itself, lead to oedema*. However, the non-appearance of oedema following the ligature of the lymphatics does not mean that the fluid circulation is not gravely disturbed by the cessation of lymph circulation in the region in question; it only means that, as a rule, it is not possible to find and to ligate a sufficient number of lymphatics and, on the other hand, that the lymphatics are — as has already been pointed out — capable of regeneration.

Reichert (1926) severed and then reunited all soft parts in the [illegible]

Drinker and his associates suspended fine silica powder in a 2·5% solution of quinine, and injected it into the lymphatics of the lower extremity of dogs. Quinine gives rise to sterile inflammation, silica to obstructive lymphangitis. By repeating this intervention several times it was possible to induce a lasting obstruction of the injected lymph vessels; the open ones dilated vicariously so that they could be found and likewise injected. Those small lymph trunks which still escaped being injected became dilated to such an extent that their valvular system was insufficient so that they were incapable of transporting lymph any longer ("valvular insufficiency" of lymph circulation).

The result was a painless, durable oedema in the hind leg with wholly intact venous circulation. The protein content of the oedema fluid increased continuously: even values of 5 g% were found. The essence of the process was this: protein is continuously filtered with the capillary filtrate through the blood capillaries. Owing to its colloid-osmotic pressure, the protein is not reabsorbed by the blood vessels, and retains also water in the interstitial space. As a result of [illegible] water metabolism and the function of the lymphatic system.

The process did not stop at oedema: there appeared further phenomena which make the experiment particularly interesting for human pathology. The dog is known to be little sensitive to bacterial infections; suppurative processes occur but rarely in this animal under normal conditions. In Drinker's oedematous dogs, however,

## CHAPTER XXII

# CHRONIC LYMPHOEDEMA AND ELEPHANTIASIS

Elephantiasis has been long known as a consequence of lymphatic obstruction; this phenomenon — entailing first swelling and then complete deformation — appears mainly in the lower extremities, less frequently in the arms or the genitalia.

The use of the term elephantiasis does not seem to be quite appropriate for the designation of the consequences of chronic lymphoedema. Elephantiasis has a wider sense: besides chronic lymphoedema, it is produced also by arteriovenous fistula, neuromatosis and lymphangiomatosis. Therefore, Allen, Barker and Hines (1946) suggest that the term elephantiasis should not be employed. In our opinion, it is, however, right to retain this plastic term, provided we always know the sense in which it is employed.

Elephantiasis is classified in different ways in the textbooks. Earlier, only "European" and "tropic" elephantiasis were mentioned, while — nowadays — quite a number of forms are distinguished. Allen and his associates, for instance, classify their 300 cases of chronic lymphoedematous elephantiasis as follows:

I. *Non-inflammatory lymphoedemas*

A) *Primary*
1. Lymphoedema praecox
2. Congenital lymphoedema
   a) hereditary or familiar (Milroy's disease)
   b) simple

B) *Secondary*
1. Malignant obliteration
2. Surgical removal of lymph nodes
3. Pressure
4. X-ray or radium irradiation

II. *Inflammatory lymphoedemas*

A) *Primary*
B) *Secondary*
1. Insufficiency of venous circulation
2. Trychophytosis
3. Systemic disease
4. Filariasis
5. Local injury or inflammation of tissues

[illegible]

the better.

[illegible]

occasions.

[illegible]

terramycin. After transitory improvement, his condition deteriorated anew.

Having been admitted to a hospital in the autumn, the patient received there penicillin-streptomycin-hyasone infusions. This therapy was twice combined with hyasone injections into the upper lip. This treatment also led only to a transitory improvement.

By December, 1955, the oedema had become permanent. it was larger in the morning and became milder in the evening. The oedema was soft and pasty.

There is no doubt that in this case we have a diffuse facial lymphoedema, in the pathogenesis of which an infection connected with the extraction of 16 teeth plays a decisive role. The cervical lymphadenitis, on the one hand, and the removal of the cervical lymph nodes, on the other, contributed to rendering the situation still worse. *The insufficiency of lymph circulation* is sure to be of a complex nature, the surprising result of the block of the stellate ganglion proves convincingly that the condition is aggravated not merely by the organic closure of the lymph vessels but also by lymphangiospasm. That the oedema increases in volume in the cold season is we think due to the fact that capillary permeability is increased by cold and partly to [illegible]

We have seen that the classification of Allen, Barker and Hines distinguishes between a primary and a secondary form of inflammatory lymphangitis. We do not regard this distinction as significant: all that primary lymphangitis means is that the nature of the infection is not clear to us.

*Noteworthy is the elephantiasis of female genitalia*, a concomitant of inflammatory processes (Trichomonas vaginalis, cervicitis).

This is due to a spreading of the inflammatory process to the lymph- [illegible] lymph trunks.

Lymphangiectasia seems to be the essential factor in congenital lymphoedema: it leads to an insufficiency of the valves, i.e. of the

erysipelas arose spontaneously in the oedematous area, with local reddening, increasing tumefaction and fever. Beside these spontaneous inflammations, experimental infections could also readily be induced.

In the further course of the process, newly-formed connective tissue *appeared in the lymphoedematous region which then began to cicatrize.* Human lymphoedema could thus completely be reproduced in animal experiments.

To what are we to attribute in human pathology the extended *and definitive stoppage of lymph circulation in the leg? It is generally* accepted that, in *tropic* elephantiasis, the lymphatics are obstructed by filariae. Certain recent reports express, however, the opinion that the matter in point is a common infection associated with filariasis which gives rise to obstructive lymphangitis.

In the majority of the *European* cases of elephantiasis *infection* can be demonstrated as the decisive genetic factor. For instance, insect-bite is frequently mentioned in the anamnesis, followed by an apparently common lymphangitis with the swelling of the inguinal lymph nodes and lymphadenitis. The visible symptoms of acute [illegible] but, after some weeks or months, [illegible] ema — [illegible] spreads later to the leg, then to the thigh, and turns chronic.

At this stage, the oedema may temporarily be diminished by keeping the patient in bed and the affected leg propped up; further — as we have ascertained — by the administration of mercurial diuretics (see the discussions concerning the therapy of lymphoedema); after some time, however, fibrosis and sclerosis of the oedematous connective tissue set in, with a permanent thickening and deformation of the extremity.

It is very characteristic for the process that — as was observed in Drinker's animal experiments — streptococcal infections occur from time to time and that, thereafter, the general condition of the patients shows marked deterioriation. This is easily understandable if it is considered that inflammation leads to the accumulation of various protein substances, i.e. that it increases the amount of stagnant proteins to be removed, and that — if in a given case open lymphatics capable of transportation still happen to have remained — the inflammatory process will lead to lymphangitis and obliteration.

In this connection, our following case might be found to be instructive (Foldi 1955).

[illegible]

tion always subsided subsequently.

In 1951, lymph nodes were observed on both sides of the neck and the upper lip remained constantly swollen afterwards. This swelling was more marked in cold

weather. The swelling of the cervical lymph nodes was thought to be of tuberculous origin, and so they were extirpated in the same year.

At the beginning of 1953, not only the lip but the entire left half of the face became tumefied without any apparent cause. After treatment with isonicotinic acid hydrazide, the swelling of the lymph nodes that had been left over after the operation almost completely disappeared, while the oedema did not show any change for the better.

[illegible]

occasions.

[illegible]

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clear to us.

*Noteworthy is the elephantiasis of female genitalia*, a concomitant of inflammatory processes (Trichomonas vaginalis, cervicitis).

This is due to a spreading of the inflammatory process to the lymphatic system of the pelvis which drains also the lymphatics of the inferior extremity; this leads to lymphadenitis and the obliteration of the lymph trunks.

Lymphangiectasia seems to be the essential factor in congenital lymphoedema: it leads to an insufficiency of the valves, i.e. of the

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... on.

... to be instruc-

...

remained constantly swollen afterwards. This swelling was more ...

B. B., female, married, 43 years old, does not remember past diseases. In February, 1953, she felt pains in the right lower part of the abdomen; her physician [illegible]

to the abdomen.

[illegible]

1. Lack of symptoms pointing to decompensation argued against cardiac oedema. It was only to the left that the heart was a finger's breadth enlarged; Gärtner's symptom was negative and the liver of normal size.

2. The possibility of hypalbuminaemic oedema could be excluded on the evidence of the physical examination. the oedema did not extend to face and eyelids and the urinar analysis gave negative result. The protein level of the serum was normal (7 5 g%)

3 Also the possibility of a venous thrombosis had to be taken into account. [illegible] fectly normal.

[illegible]

the inflammatory process may sometimes spread over from the lymphatics of the [illegible] inflammation had in this case extended also cranially, as — besides the oedema of the inferior extremities — also the abdominal wall and the thoracic skin were found to be oedematous

[illegible] diuretics, we performed small incisions; great amounts of fluid gushed forth from the wounds, and these interventions brought temporary relief

The patient became increasingly cachectic and died soon.

[illegible]

According to Holman, McSwain and Beal (1944) amputation of the mamma is followed by the development of lymphoedema or elephantiasis only in cases of postoperative inflammations or postoperative irradiation with X-rays. The extirpation of the lymph nodes is probably never complete and, besides, lymphatics and lymph are known to be capable of regeneration; if, however, the few remaining unoccluded lymphatics become obstructed by cicatrization owing to lymphangitis or

lymph circulation. Lymphoedema is really congenital in some cases; oedema is already present at birth, while it appears in childhood in other cases. Secondary infection are sometimes added to the existing disease in such cases; the situation is then aggravated in the manner discussed above, so that it is sometimes almost impossible to ascertain whether one is dealing with a late appearance of congenital lymphoedema with secondary infection, or with acquired elephantiasis with primary infection, the first infection is often overlooked.

Two forms of congenital lymphoedema are generally distinguished. The rarer form is Milroy's disease (1928) which is inherited, while heredity plays no part in the other form.

Bloom reported in his work, published in 1941, on a family 6 of whose 37 members, belonging to 4 generations, suffered from congenital lymphoedema with ptosis of the upper eye-lids: 3 members of the family had ptosis alone. The literature contains one case of a newborn child who had generalized oedema with ascites and hydrothorax. Histologica[illegible] Of interest is the [illegible] of a newborn child was oedematous; later, erysipelas appeared repeatedly in the extremity. This, too, is an example to show that non-inflammatory and inflammatory forms of lymphoedema may appear simultaneously.

Servelle, Albeaux–Fernet, Laborde, Chabot and Rouguelle (1957) discussed the so-called Klippel–Trenaunay syndrome which reveals the following essential features: the extremity is oedematous, its soft parts and bones are elongated; the disease is accompanied by a disturbance in the development of the veins. Lymphographic examinations show that this syndrome involves different developmental anomalies of both the veins and the lymph vessels.

Recent publications justify the conclusion that trauma may be followed by the appearance of lymphoedema after some time. It is possible that, in such cases, temporary lymphangiospasm constitutes the first link in the chain of pathological happenings.

That form of lymphoedema which is termed *lymphoedema praecox* by Allen, Barker and Hines appears in young girls during puberty. Its cause is unknown. Experience shows that the situation grows worse during menstruation so that hormonal disturbances are suspected as the aetiological factor. There are authors who think it possible that the lymphatics of the pelvis cannot keep pace with the sudden rapid development of the interior sexual organs which might lead to insufficiency of lymph circulation in the lower limbs. Infections also may impair the situation, and it is probably wrong to classify this form of lymphoedema schematically as "non-inflammatory".

Lymphoedema may also occur if some *malignant process* extensively infiltrates the lymphatics and lymph nodes of a region, or if the lymph nodes are radically extirpated. We were in a position to observe such a case (Földi and Gergely 1955, unpublished).

irradiation, the blocking of lymph flow may become complete. On the other hand, Kettle (1957) has recently drawn attention to the fact that lymphangiosarcoma is comparatively frequently seen to arise in the lymphoedema of the arm, provoked by mammary amputation: the risk of such a tumour developing is greatest in those who have survived radical mastectomy by at least 5 years and who may thus be regarded as having a very good chance of cure of their mammary carcinoma.



*Fig. 252.* Phlebography of the patient represented in Fig. 250. Normal filling of veins

Elephantiasis sometimes develops in *causalgic extremities*. This may be explained by immobility caused by pains (patients often leave their arm pendant.) It is known that lymph flow in the extremities is maintained by the contraction of muscles; a complete cessation of contractions leads to the cessation of lymphatic drainage.

Lymphoedema develops sometimes also in the paralysed extremities of *apoplectic* persons (Luhan 1936; Takáts and Evoy 1950; Lowen-



*Fig 250* Extended lymphoedema. Impression of fingers on thigh and trunk



*Fig. 251.* Right-hand inguinal area of the female patient represented in Fig. 250 Lymphangiectasis visible

Results that can be expected from mercurial diuretics are considerably less favourable in the advanced stage when the picture is already dominated by fibrosis or sclerosis. But novurit produces a certain effect even in these cases, and can therefore be recommended for the purposes of a preoperative therapy.

*Operation* is the only procedure that can be considered in cases of elephantiasis of the leg connected with its excessive deformation. Not only cosmetic considerations require surgical intervention but also the effort to restore the use of his leg to the patient who has become completely or almost completely unable to walk.

The first recorded surgical treatment of elephantiasis is that applied by Lisfranc (cit. Servelle 1952) at the beginning of the 19th century; this author recommended the scarification of skin. This method has now only historic interest, as also the procedure of Carnochan (cit. Servelle 1952), who — in order to decrease the oedema — tied off the external iliac artery; he was led by the consideration that the *oedema would diminish if the blood supply of the extremity were reduced.*

Handley described in 1909 a new method for the operation of [illegible]

Some years after Handley, Walther (cit. Servelle 1952) tried to apply a draining tube, but this procedure did not yield the desired result either.

Other authors tried to induce lymph drainage by establishing a communication between the diseased area and the system of lymph vessels situated in the deeper layers of the lower extremity. With this in view, Lanz (1911) resected a piece of the superficial aponeurosis and trephined the bone, while a piece of the fascia lata and the elephantiac connective tissue was excised by Kondoleon (1912). These operations [illegible]

the bare muscles. (Servelle 1952.) Servelle describes this operation, termed *lymphangiectomia superficialis totalis*, as follows:

"The operation is carried out in two sittings. We operate first the outside of the extremity, and after 2 to 3 months its inside. The operative area is made bloodless by means of Esmarch's bandage. In the first operation, the incision runs from the greater trochanter to the outside ankle. Only the skin is cut and lifted from the connective tissue forwards and backwards as far as the middle line. Thereafter, we make an anterior and a posterior incision into the diseased connective tissue as well as into the superficial aponeurosis, and excise the whole in a single block. The two skin flaps are then reunited . . .

berg 1952); it is due to the co-operation of several factors. The first to be mentioned is that — according to Gömöri, Kisfaludy and Urai (1948) — the permeability of the blood capillaries increases in paralysed extremities. To the so increased task is then added the cessation of lymph drainage in the motionless limb.

Hagentorn described (1904) a rare form of elephantiasis under the name of "Elephantiasis tuberosa". He published the case of a 42-year old woman whose disease had begun 7 years before with erysipelas on the leg. Some time after the subsidence of the acute inflammatory phenomena there arose oedema which gradually extended to the femur in the course of 3 years. It resulted in elephantiasis which differed from the usual forms in the development of walnut-sized nodes; the skin was pink and cyanotic, and excoriations formed on it from which 3 litres of a yellowish, malodorous fluid was seeping every day. The specific gravity of this fluid was about 1011, its protein content amounted to 0·37%.

The diseased extremity was amputated, with the result, however, that the process appeared on the homolateral labia of the vulva.

The *therapy* of elephantiasis constitutes an exceedingly difficult problem. Lowenberg underlines the importance of prophylaxis in thrombophlebitis; he attaches importance to the application of anticoagulants (heparin) because he thinks that they prevent a thrombosis in the lymphatics.

Particularly in lymphoedema of inflammatory origin it is worth while to try to infiltrate the sympathetic trunk (or the sympathetic fibres around the femoral artery) with procaine. If this gives a satisfactory result, it may be followed by sympathectomy. We have mentioned that incipient oedema may transitorily be reduced also by simple means (keeping the patient in bed, propping up of the leg, mild massage). We want to emphasize the value of mercurial diuretics. We described, in the chapter on physiology, the experiments which proved the lymphagogue effect of novurit. It is sometimes possible to obtain striking results with 1—2 novurit injections. Just as in cardiac oedema, diuresis increases, the skin of the extremity gets wrinkled and its circumference may diminish by several centimetres. By a regular administration of novurit (several times per week, and if need be, also in small daily doses), the life of the oedematous patient may be made tolerable for a long time.

It is, of course, not merely in cases of the lymphoedema of the lower extremities that diuretics can be applied. We found that the huge facial oedema which occur after the so-called block dissections in metastatic laryngeal carcinomas (the cervical lymph nodes are radically extirpated in this operation) respond likewise to novurit. In one of our cases we employed it with satisfactory result in a voluminous postoperative lymphoedema of the upper eyelid. Hyaluronidase-iontophoresis has recently been found to give good therapeutical results in cases of lymphoedema; so far, we have had no occasion to test this method.

In cases of an *arteriovenous* fistula the veins are dilated, whereas the arteriovenous difference of oxygen is very low. That also arteriovenous fistula may be taken into account from the viewpoint of differential diagnosis is due to the observation that, in its congenital forms, the extremity is strongly enlarged both lengthwise and crosswise.

Difficulties of differentiation may arise in connection with the picture produced by disturbances of the *venous circulation*. Post-thrombophlebitic conditions may assume the form of lymphoedematous elephantiasis to such an extent that it is possible to speak of "post-thrombophlebitic elephantiasis" as distinguished from "lymphogenous elephantiasis". Of course, a disturbance of the lymph vascular apparatus plays a role also in the pathogenesis of the latter disease. We have seen that thrombophlebitis may be accompanied by lymphangiospasm, lymphangitis and perilymphangitis, i.e. that one is dealing with a mechanical insufficiency of the lymph circulation. The consequences are naturally the same as in the primary disease of the lymphatic system. If thrombophlebitis connected with violent clinical symptoms is mentioned in the case history, or if varicosis is diagnosed, it is comparatively easy to distinguish the case from the pure, "lymphogenous form", but there are cases where both are absent. It should be noted that no ulcus cruris has been observed in cases of pure "lymphogenous" elephantiasis.



*Fig. 254* Lymphangiographic representation of a lymphangioma and the efferent lymphatics issuing from it (a case of Földi and Gergely)

The practical importance of the question lies in the fact that, when the patient is operated upon, all superficial veins are also extirpated. This, however, is absolutely counterindicated if the circulation in the deeper veins is not perfectly faultless. Therefore, it should be observed as a strict rule that preoperative venography must invariably be performed: operation is only permissible if the veins of the deeper layers present a normal picture. A radical extirpation of the superficial

The inner side is operated after 2 to 3 months, when the wound of the *preceding operation has completely healed*".

This operation leads allegedly to excellent and lasting results. The elephantiac tissues are radically extirpated; complete extirpation makes it *possible for the lymphatics of the skin to grow into the lymphatic system of the musculature in the deeper layers of the leg.*



*Fig 253.* Visualisation of the lymph vessels prior to lymphangiography by subcutaneous injection of Evans-blue

According to Servelle, appropriate preoperative preparation is very *important. The patient must be kept in bed before the operation;* the [illegible]ption of [illegible]ntioned [illegible]

It is also from a practical point of view that great importance attaches to the question of *differential diagnosis.* It has been noted — apart from lymphoedema — other factors also may induce elephantiasis. It may, for instance, arise as a consequence of any lasting oedema and very seldom even of cardiac oedema. We have seen that, in oedema, the disturbance of the lymph circulation also plays a role in these cases. However, elephantíasis arises always bilaterally in such cases, while true elephantiasis is always unilateral. Myxoedema, too, may be considered: this induces likewise bilateral oedema.

patent-blue solution (dissolved in distilled water) between the toes and then make a transversal incision into the skin on the dorsum of the foot; a contrast medium is then injected into the blue-stained lymphatics. Kaindl, Mannheimer, Polsterer and Thurnher (1957a, b) employ prontosil rubrum for the visualisation of the lymphatics. The lymphatics of lymphoedematous patients are dilated and show a sinuous course so that the stain can be followed both in a lateral and in a retrograde direction, they were never able to observe this phenomenon in the lymphatics of normal limbs. We (Gergely, Zsebők and Földi 1956; Gergely and Zsebők 1956) employed this method with success. Leenhardt and Colin (1957), as well as Hillander, Reilly and Burrows (1956), inject radioactive material intralymphatically.

Let us add that we (*Földi and Gergely 1955, unpublished*) succeeded in making markedly visible not only the lymphangiomatic vesicle but also its efferent lymphatics by the puncture of a lymphangioma on the thigh into which a 70% solution of ioduron was injected (*Fig. 251*).

Let us also refer to those cases of congenital anomalies in which the elephantiac lymphangiectasis, if punctured, yields — instead of lymph of a peripheral character — *a yellowish-white fluid rich in fat: chyle.* In some cases of elephantiasis a lymph fistula is encountered in the lower extremity from which chyle containing an enormous amount of fat is emptied. Munk and Rosenstein, *for instance*, recovered 60 per cent of the ingested fat through the lymph fistula.

Recently, we too observed (Földi, Ránky and Koltay 1954, unpublished) a case of this kind.

A female patient, 33 years of age, whose history mentioned an erysipelas that had occurred 14 years before, complained that her right leg had been gradually swelling up for the last few months. She noticed the appearance of vesicles on the anterior and the inner surface of her lower limb during the last weeks (Fig. 255). As a result of the examination typical lymphoedema was diagnosed, the vesicles corresponded to lymphangiectasies. The right inguinal bend was strongly swollen and painless lymphatics were palpable there.

TABLE 72

| | Lymph fat | Serum fat |
|---|---|---|
| Before the meal | 215 mg% | 625 mg% |
| 1 h after ingesting 100 g of fat | 1470 « | 680 « |
| 2 « « « 100 g « « | 6125 « | 670 « |
| 3 « « « 100 g « « | 6550 « | 670 « |
| 4 « « « 100 g « « | 3850 « | 670 « |
| 5 « « « 100 g « « | 3450 « | 770 « |
| 6 « « « 100 g « « | 3450 « | 980 » |

venous network leads to the degeneration of the extremity if the venous system of the deeper layers is incompetent. This is to say that we are as yet unable to heal post-thrombophlebitic elephantiasis by surgical intervention.

In certain cases tremendously dilated lymphatics are subcutaneously visible and palpable and can sometimes be punctured. Servelle

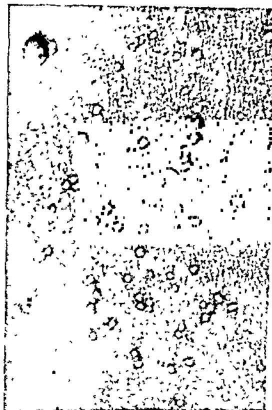


*Fig. 255.* Lymphangiectasies on skin (a case of Földi and Ránky)

was able to withdraw 1500 ml of lymph in one of his cases. If we inject radio-opaque substance into the lymph vessels, the roentgenogram will reveal dilated, tortuous and varicose lymphatics. In other cases no such superficial lymphangiectasies are present but dilated lymphatics are sometimes to be seen beside the saphenous vein that has been isolated for the purposes of venography.

Kinmonth, Taylor and Harper (1955) perform lymphangiography in human subjects in the following manner: they inject isotonic (1%)

## CHAPTER XXIII

# REGENERATION OF LYMPHATICS AND LYMPH NODES

We have already emphasized the extraordinary difficulty of inducing chronic lymphoedema in animal experiments; even if one succeeds in producing lymphoedema by means of tying off or extirpating the lymph vessels and lymph nodes, the oedema will disappear, as a rule, after a few days. It is probable that, generally, not all lymphatics and lymph nodes are ligated; it is moreover known that preformed lymphatico-venous anastomoses may open or new ones be formed, and that the possibility of a regeneration of the lymph nodes and vessels also exists.

Numerous authors have dealt with the problem of regeneration since the end of the last century. As regards the regeneration of lymph nodes, Bayer (1885, 1886, 1891, 1895), Hammerschlag (1908), Zehnder (1890), Ritter (1900, 1905, 1907), Groot (1912), Reddingius (1912) and others observed the regeneration of lymph nodes partly in experiments where the lymph nodes of animals were extirpated, partly in humans whose lymph nodes were destroyed by carcinoma. Furuta (1948) suggests that a regeneration of lymph nodes after their complete extirpation occurs only in young animals. The picture of the new lymph nodes is identical with that of embryonal morphogenesis.

A number of authors maintain that no new lymph nodes are developed to replace those extirpated or destroyed (Meyer 1906; Ottaviani and Cavalli 1933, etc.). These contradictions have been explained by the experiments of Rouvière and Valette (1937) who demonstrated that, while radically extirpated lymph nodes do not regenerate, from a small unremoved piece of a lymph node a new one may develop.

Some years ago, Wachtel (1949) concerned himself with the question of the regeneration of lymph nodes. He does not think that the extirpated popliteal lymph node of dogs is capable of regeneration: what happens is a possible restoration of lymph circulation by the growth of new lymphatics.

As regards the regeneration of lymphatics, it seems to be quite certain that these do regenerate in the area of operative traumas. According to Rouvière and Valette, it is not the regeneration of the lymph vessels which is the decisive factor in the restoration of lymph circulation after a ligation of the lymphatics: the decisive factor is a dilatation of those preformed vessels which have remained unimpaired. Worthy of note is the observation of Van der Brenk (1957) who found that ionizing radiation inhibited lymphatic regeneration in mammals.

The punction of the lymphangiectasies yielded a fluid containing 5·1 g% of protein. With a view to ascertaining whether a communication existed between the congested lymphatic system of the right lower extremity and the chyle vessels, we kept the patient on a fat-rich diet with the result as shown in Table 72.

These figures make it clear that in this case a communication did exist between the chyle vessels and the lymphatics of the oedematous extremity.

We want to mention, in conclusion, that Taylor, Kinmonth, Rollinson and Rotblat (1957) recently discussed the absorption from lymphoedematous areas of plasma proteins labelled with radioactive iodine and found its rate to be slower than the normal.

## BIBLIOGRAPHY*


Aagard, O. C. (1925), Les vaisseaux lymphatiques du coeur chez l'homme et chez les mammifères. Levin & Munksgaard, Copenhagen.

Abbott, W. E., S. Levey, R. C. Foreman, H. Krieger & W. D. Holden (1952). The danger of administering parenterally fluids by hypodermoclysis. Surgery 32, 305

Abdou, I. A., W. O. Reinhardt & H. Tarver (1952). Plasma protein. III. The equilibrium between the blood and lymph protein J. Biol. Chem 194, 15.

Abdou, I A. & H Tarver (1950), Plasma protein. I. Loss from circulation and catabolism to carbon dioxide J Biol Chem. 190, 769

Abdou, I. A & H. Tarver (1951), Plasma protein II. Relation between circulating and tissue protein. J. Biol. Chem. 190, 781.

Abe, Y. (1925a). Untersuchungen uber die Eigenschaften und die Entstehung der Lymphe. IX. Wirkung der spezifischen Lymphagogen auf den Pfortaderdruck. Biochem Ztschr. 165, 261

Abe, Y (1925b), Untersuchungen über die Eigenschaften und die Entstehung der Lymphe X. Untersuchungen über den Einfluß der spezifischen Lymphagogen auf die Gefäße überlebender Organe Biochem Ztschr 165, 277.

Abe, Y. (1925c). Untersuchungen uber die Eigenschaften und die Entstehung der Lymphe XI Die Beeinflussung der Lebertätigkeit durch die spezifischen Lymphagogen, nachgewiesen an der Acetonbildung Biochem. Ztschr. 165, 312

Abe, Y. (1925d), Untersuchungen uber die Eigenschaften und die Entstehung der Lymphe XII Physikalisch-chemische Untersuchungen über die Beeinflussung des Blutes durch Zusatz von spezifischen Lymphagogis bei Durchstromung durch die Leber Biochem Ztschr 165, 323

Abel, J J, L G Rowntree & B. B Turner (1913/14), On the removal of diffusible substances from the circulating blood of living animals by dialysis. J. Pharmacol 5, 625

Abel, J J, & B B Turner (1910), On the influence of the lymph hearts upon the action of convulsant drugs in cardiectomized frogs J Pharmacol. and Exp Therap 2, 167

Abrikosov, A (1929), Über »Splenomykosen« und »Mykotische Splenomegalien« Virch Arch 272, 593

Achard, C, M Bariéty & A. Wilm (1932), Thrombose veineuse coronarienne avec infarctus du myocarde au cours d'une bronchopneumonie. Bull. et mém Soc méd d'hôp. de Paris 48, 1410

Adams, W. S, R H Saunders & J S Lawrence (1945), Output of lymphocytes in cats including studies on thoracic duct lymph and peripheral blood Am. J Physiol 144, 297

Addis, T (1931/32), Proteinuria and cylindruria Proc California Acad Med 38

Addis, T (1945), Renal degenerations due to protein reabsorption by the kidney Stanford Med Bull. 3, 67

Addison, T. (1855), On the Constitutional and Local Effects of Disease of the Suprarenal Capsules Highley, London

Adler, J & S J Meltzer (1896), Experimental contribution to the study of the path by which fluids are carried from the peritoneal cavity into the circulation J Exp Med 1, 482

Adolph, E F & M J Lepore (1931), How intravenous infusions modify water contents of tissues. Proc. Soc. Exp Biol. Med 28, 963


* For translation and or transliteration of the works of Soviet and Russian authors see page 818

Asher, L. & W. J. Gies (1900), Untersuchungen über die Eigenschaften und die Entstehung der Lymphe. III. Mitt. *Über den Einfluß von Protoplasmagiften* auf die Lymphbildung. Ztschr. Biol. 22, 180.

Asellius, G. (1627), De Lacteibus sive lacteis venis Quarto Vasorum Mesaroicum genere novo invente Gasp. Asellii Cremonensis Antomici Ticiensis Qua Sententiae Anatomicae multae, nel perperam receptae illustrantur. Mediolani, apud Jo. Baptistam Bidellium

Ashburn, E. L., K. M. Endicott, F. S. Daft & R. D. Lillie (1947), Nonportal distribution of trabeculas in dietary cirrhosis of rats and in carbon tetrachloride cirrhosis of rats and guinea-pigs. Am. J. Path. 23, 159

Askanazy (1904), Zur Physiologie und Pathologie der Plexus chorioidei. Verh. d. deutsch. path. Gesselsch.

Aub, J. C., A. M. *Brues, S. S. Kety, I. T. Nathanson, A. Pope*, L. L. Robbins & P. C. Zamecnik (1945), Toxic factors in experimental traumatic shock; effects *of intravenous injection of effusion* from ischemic muscle. J. Clin. Invest. 24, 845

Augustine, D. L. & C. K. Drinker (1935), The migration of mikrofilariae (Dirofilaria immitis) from the blood vessels to the lymphatics. Trans. Roy. Soc. Trop. Med. & Hygiene, 29, 303

Axenfeld, H. & K. Braas (1912/13), Klinische und bioptische Untersuchungen über den sogenannten Ikterus katarrhalis Frankfurter Ztschr. Pathol. 57, 147.

Babes & Zeiss (1877), Mikroskopische Untersuchungen über den Bau der Schilddrüse. Straßburg

*Babics, A., M. Földi, F. Rényi-Vámos, Gy. Rombányi*, I. Rusznyák & Gy. Szabó (1954a), A Disse-tér és a máj nyirokérrendszerre (Disse's space and the lymph vascular system of the liver) Magyar. Belorv. Arch. (Hungarian), 7, 7.

Babics, A., M. Földi, F. Rényi-Vámos, Gy. Rombányi, I. Rusznyák & Gy. Szabó (1954b), A máj nyirokkeringésének jelentősége choledochus elzáródásában és cholangitisben (Significance of hepatic lymph circulation in the obstruction of the choledochus and in cholangitis) Magyar Belorv. Arch. (Hungarian), 7, 261

Babics, A., M. Földi, F. Rényi-Vámos, G. Rombányi, I. Rusznyák & Gy. Szabó (1955), Das Lymphgefäßsystem der Leber und seine pathologische Bedeutung *Acta Med. Hung. 7, 261.*

Babics, A. & F. Rényi-Vámos, (1950), A vese üregrendszerének pathophysiológiája és műtétei (Pathophysiology and operations of the renal cavities). (Hungarian), Akadémiai Kiadó, Budapest.

Babics, A. & F. Rényi-Vámos (1952a), Die ascendierende Pyelonephritis Acta Med. Hung. 3, 15

Babics, A. & F. Rényi-Vámos (1952b), A vesepusztulás elmélete és klinikuma (Theory and clinical picture of renal atrophy) (*Hungarian*), Akadémiai Kiadó, Budapest

Bagdy, D., M. *Földi, M. Gerendás*, I. Rusznyák, & Gy. *Szabó* (1950), Hyaluronidase-heparin antagonizmus (Antagonism between hyaluronidase and heparin) Orv. Hetilap (Hungarian), 91, 327

Baggenstoss, A. H. (1957), Pathological Anatomy of Hepatitis, J. Am. Med Assoc. 165, 1099

Bainbridge, F. A. (1901), Observations on the lymph flow from the submaxillary gland of dog. J. Physiol. 26, 79.

*Bainbridge, F. A.* (1902), On the *formation of* lymph by the *liver* J. Physiol. 28, 204

Baitsell, G. (1916), The origin and structure of a *fibrosis tissue formed in* wound healing J. Exp. Med. 23, 739

Baitsell, G. & K. E. Mason (1930), The origin of a fibrosis tissue arising in the testis of the guinea-pig following experimental tuberculosis Am. Rev. Tbc 21, 592

Baker, R. D. (1932/33), The cellular content of chyle in relation to lymphoid tissue and fat transportation Anat. Record. 55, 207.

Balankura, N. R. (1951), Development of the *mammalian lymph* system. Nature, 168, 196

Afanasiew, N. (1868), Ueber den Anfang der Lymphgefäße in den serösen Hauten. Virchows Arch 44, 37.

Alburn, H. E. & R W. Whitley (1954), Anionic hyaluronidase inhibitors Federation Proc. 13, 330.

Alfejew, S. (1926), Über die embryonale Histogenese der kollagenen und reticulären Fasern des Bindegewebes bei Säugetieren. Ztschr. Zellforsch. u. mikr. Anat 3, 149.

Allen, E. V., N. W Barker & E. A. Hines (1946), Peripheral vascular diseases. Saunders, Philadelphia & London.

Allen, L. (1936), The peritoneal stomata. Anat. Record 67, 89.

Allen, L. & E. Vogt (1937), A mechanism of lymphatic absorption from serous cavities. Am J Physiol. 119, 776.

Allen, T. H & P. D. Orahovats (1950), Combination of toluidine dye isomers with plasma albumin Am J. Physiol 161, 473.

Alrich, E. M. (1944), Studies on burns; observations on vasoconstrictor substance in lymph from burned area Surgery 15, 908.

Сб. работ к XV лет Белорусск гос мед ин-та 73

Angevine, R. W. (1929), Fat excretion, excretion by Thyry-Vella fistulas J. Biol Chem. 82, 559.

Anitschkow, N. (1924), Zur Frage der Verteilung intravenos eingefuhrter Kolloidsubstanzen im Organismus. Klin. Wschr. 3, 1729.

Anitschkow, N. (1929), Einige Untersuchungen uber die Speicherung von Vitalfarbstoffen und Aufschwemmungen im Organismus Virchows Arch 275, 93.

Apitz, K (1940), Die neuen Anschauungen von Plasmacytom des Knochenmarks, dem sog. multiplen Myelom. Klin Wschr. 19, 1025.

Armentano, L , A. Bentsáth, I. Rusznyák & A. Szentgyorgyi (1936), A flavoncsoportokba tartozó anyagok befolyása a capillarisok permeabilitására (Effect of flavonoid substances on capillary permeability). Orv Hetilap (Hungarian), 80, 935.

Arnold, J. (1890), Die Geschicke des eingeatmeten Metallstaubes im Korper Zieglers Beitr. 8, 1

384, 385.

hemical

льных

hagen.

Munks-

Ztschr.

Biol. 11,

Asher, L. (1898), Untersuchungen uber die Eigenschaften und die Entstehung der Lymphe. II Mitteilung. Ueber Beziehungen zwischen Organtatigkeit und Lymphbildung. Ztschr. Biol. 19, 261.

lymphagogue de la propeptone.

g der Lymphbildung und der

4, 999.

ungen uber die Entstehung und die Eigenschaften der Lymphe. Ztschr. Biol. 18, 154

Asher, L. & F. W. Busch (1900), Untersuchungen uber die Eigenschaften und die Entstehung der Lymphe. IV. Mitt. Ztschr. Biol. 22, 333.

Bauer, W. & W. Richards Jr. (1928). Vaso-dilator action of acetates. J. Physiol. 66, 371.
Baum, H. (1911). Können Lymphgefäße direkt in Venen einmünden? Anat. Anz. 39, 593
Baum, H. (1916/17). Können Lymphgefäße direkt ins Venensystem einmünden? Anat. Anz. 49, 407.
Baum, H. (1917). Lymphgefäßsystem des Rindes. Hirschwald, Berlin.
Baum, H. (1918). Lymphgefäßsystem des Hundes. Hirschwald, Berlin.
Baum, H. (1926). Die Lymphgefäße der Lunge des Pferdes, Rindes, Hundes und Schweines Ztschr. Anat. u. Entwickl. 28, 711.
Baum, H. (1928). Lymphgefäßsystem des Pferdes Berlin.
Baum, H. & S. Kikara (1929). Untersuchungen über den Bau der Lymphgefäße und den Einfluß des Lebensalters auf diese Z. mikr.-anat. Forsch. 18, 159.
Baum, H & Trautmann (1933). Das Lymphgefäßsystem der Säugetiere. In Bock—Göppert—Kallius—Lubosch, Handbuch der vergleichenden Anatomie der Wirbeltiere. VI Leipzig.
Bayer, C. (1885). Über Regeneration und Neubildung der Lymphdrüsen Ztschr. Heilkunde 6, 105
Bayer, C. (1886). Weitere Beiträge zur Lehre von der Regeneration und Neubildung der Lymphdrüsen. Ztschr. Heilkunden 7, 423.
Bayer, C. (1891). Über die Bedeutung des Fettgewebes für den Aufbau lymphatischer Neubildungen. Ztschr. Heilkunde 12, 517
Bayer, C. (1895). Altes und Neues über kranke Lymphdrüsen. Langenbecks Archiv 49, 637.
Bayliss, W. M. & E. H. Starling (1894). Observations on venous pressures and their relationship to capillary pressures J. Physiol. 16, 159
Báhnt, P & Gy. Benkő (1948). A nátrium és chlor megoszlása a vérserum és a savós üregek folyadékgyülemei, valamint a liquor cerebrospinális között (Distribution of sodium and chlorine between the accumulated fluids of the serum and the serous cavities on the one hand and the cerebrospinal fluid on the other). Magyar Belorv. Arch. (Hungarian), I, 97.
Beams, A J. & O Harmos (1949). Diffuse progressive interstitial fibrosis of the lungs. Am J Med 7, 425.
Beazell, J. M., C. K Schmidt & A. C Ivy (1937). The effect of heat on blood and lymph flow from the gastrointestinal tract. Am J Physiol. 119, 197.
Beck, C S (1924). A study of lymph pressure. Bull Johns Hopkins Hosp 35, 206.
Beckmann, K., E. Bass, R Dürr & G Droshn (1925). Stoffwechsel- und Blutveränderungen nach Nierenexstirpation III. Mitteilung Eiweißverschiebung und Änderungen des chemisch-physikalischen Verhaltens des Plasmaeiweißes. Z. ges. exp. Med 53, 420
Becht, F C & A B Luckhardt (1916). The origin of the antibodies of the lymph. Am J Physiol 40, 366
Beecher, H K (1937). Adjustment of the flow of tissue fluid in the presence of localized sustained heigh venous pressure as found with varices of the great saphenous system during walking. J. Clin. Investigation 16, 733.
Beecher, H K (1936). The independent control of the capillary circulation on mammal Scand Arch Physiol. 73, 1
Beecher H K (1943). Resuscitation and sedation of patients with burns which include the airway Annals of Surgery 117, 825.
Beecher, H K., M E Field & A Krogh (1936). A method of measuring venous pressure in the human leg during walking. Skand. Arch. Physiol. 73, 7.
Beiglböck W (1944). Zur Frage der »Hepatitis epidemica«. Ztschr. klin. Med 143, 530
Beiglböck W & H Junk (1937). Der Muskeltonus und seine Beziehungen zum peripheren Kreislauf (Ergebnisse von Messungen des Muskelinnendruckes nach Hendestone) Ztschr klin Med. 131, 241.
Beilinsohn, A V (1929). Effect of Bayer 205 (Germanin) on action of trypsin. J Exp Biol & Med 11, 52

Banks, H. H., A. M. Seligman & J. Fine (1949), The effect of hyaluronidase on the absorption of parenterally administered radioactive plasma proteins in the dog. J. Clin. Invest. 28, 548.

Baráth, E. (1930), Klinische Beiträge zum Verhalten des onkotischen (kolloidosmotischen) Druckes I. Der onkotische Druck im Serum des arteriellen und venösen Blutes bei Kranken mit und ohne Storungen im Wasserhaushalt Ztschr. klin. Med 114, 702.

... permeability during haemor-

... involved in the concentra-

Barcroft, J. & T. Kato (1915), Effects of functional activity in striated muscle and the submaxillary gland. Phil. Trans Roy. Soc. London B 207, 149

Bardy, H. (1914/15), Über Hemmung inflammatorischer Symptome. Skand Arch Physiol. 32, 198.

Bargebuhr, A. (1895), Chylose und chyliforme Ergusse in Pleura und Pericardialraum. Dtsch. Arch klin. Med. 54, 410

Bargen, J A & C F. Dixon (1935), Regional ileitis Proc. Staff Meet. Mayo. Clin. 10, 814.

... in dogs

... snake

... the migra-

Barry, D. T. (1923), Pulmonary edema and congestion in the heart-lung preparation. J Physiol. 57, 368

Bartels, P. (1904), Über die Lymphgefäße des Pankreas. I. Über lymphatische Verbindungen zwischen Duodenum und Pankreas beim Hunde Arch. f Anat u. Physiol. Anatomische Abt. 229.

Bartels, P. (1906), Über die Lymphgefäße des Pankreas. II. Das feinere Verhalten *der lymphatischen Verbindungen zwischen Pankreas u. Duodenum* Arch. f Anat. u. Physiol. Anatomische Abt. 250

Bartels, P. (1907), Über die Lymphgefäße des Pankreas III. Die regionären Drusen des Pankreas beim Menschen. Arch f Anat. u Physiol Anatomische Abt. 267.

Bartels, P. (1909), Das Lymphgefäßsystem Handbuch d. Anatomie d Menschen. G Fischer, Jena.

Bartolinus, T. (1653), Vasa lymphatica nuper Hafniae in Animalibus inventa, et Hepatis exsequiae. Hafniae.

Bartolinus, T. (1657), De lacteis thoracis in homine brutisque nuperrime observatis. Hafniae.

Basler, A. (1914), Untersuchungen über den Druck in den kleinsten Blutgefäßen *der menschlichen Haut. II. Mitt. Pflugers Arch. 157,* 345.

Bauer, R. O, W. H Graves Jr, C. S. Hill & K. J. Milch (1954), Acute experimental hemorrhagic shock in the dog treated with subcutaneous hyaluronidase dextran solution. Proc Soc. Exp. Biol. Med. 87, 341.

Blalock, A. & G. W. Duncan (1942), Traumatic shock-consideration of several types of injuries. Surg. Gynec. & Obst. 75, 401.
Blalock, A., A., C. S. Robinson, R. S. Cunningham & M. E. Gray (1937), Experimental studies on lymphatic blockade. Arch. Surg. 34, 1049.
Bloom, B., I. L. Chaikoff & W. O. Reinhardt (1951), Intestinal lymph as pathway of transport of absorbed fatty acids of different chain lengths. Am. J. Physiol. 167, 450.
Bloom, B., I. L. Chaikoff, W. O. Reinhardt, C. Entenman & W. G. Dauben (1950), The quantitative significance of the lymphatic pathway in transport of absorbed fatty acids. J. Biol. Chem. 184, 1.
Bloom, D. (1941), Hereditary lymphedema (Nonne—Milroy—Meige). Report of family with hereditary lymphedema associated with ptosis of the eyelid in several generations. N. Y. State. J. Med. 41, 856.
Bloom, W. M. (1923), The role of the lymphatics in the absorption of the bile pigment from the liver in early obstructive jaundice. Bull. Johns Hopkins Hosp. 34, 316.
Bockus, H. L. (1946), Gastro-enterology. Saunders, Philadelphia—London.
Boechat, P. A. (1873), Recherches sur la structure normale du corps thyreoïde. Delahaye, Paris.
Boerhaave, H. (1738), Oratio de usu ratiocinii mechanici in medicina, habita 24 Sept. 1702. In Opusc. omnia. Hagae-Comitis.
Boerhaave, H. (1742), Praelectiones academicae in proprias institutiones rei medicae. Ed. Alb. Haller, Taurini.
Bofill-Deulofeu, J. (1932), Die agrophilen Faserstrukturen in mesenchymalen Gewebekulturen von verschiedener Herkunft und von verschiedener Wachstumgeschwindigkeit. Z. Zellforsch. u. mikr. Anat. 14, 744.
Bogdanus, M. (1654), Insidiae structae Cl. V. Thomae Bartholini . . . Vasis Lymphaticis ab Olao Rudbeckio Sueco in suis Ductibus hepaticis et vasis glandularum serosis Arosiae exitis. Frankfurt.
Bogdanus, M. (1654), Apologia pro vasis lymphaticis Th. Bartholini a M. Bogdano contra insidias secundo scriptos ab Olao Rudbeck. Hafniae.
Boit, H. (1913), Über Herzbeutelresorption. Beitr. klin. Chir. 86, 150.
Bollman, J. L. (1951a), Studies of hepatic lymphatics. Liver Injury. Trans. 9th Conf. J. Macy Found. New York.
Bollman, J. L. (1951b), Liver lymph and intestinal lymph in experimental cirrhosis and ascites. J. A. M. A. 145, 1173.
Bollman, J. L. (1953), Extravascular diffusion of dextran from blood. J. Lab. Clin. Med. 41, 421.
Bollman, J. L., J. C. Cain & J. H. Grindlay (1948), Techniques for the collection of lymph from the liver, small intestine and thoracic duct of the rat. J. Lab. Clin. Med. 33, 1349.
Bollman, J. L., E. V. Flock, J. C. Caine & J. H. Grindlay (1950), Lipids of lymph following feeding of fat: an experimental study. Am. J. Physiol. 163, 41.
Bollman, J. L. & E. V. Flock (1951), Cholesterol in intestinal and hepatic lymph in the rat. Am. J. Physiol. 164, 480.
Bolton, C. (1921), Absorption from the peritoneal cavity. J. Path. 24, 429.
Bolton, C. & W. G. Bernard (1931), The pathological occurrences in the liver in experimental venous stagnation. J. Path. & Bacter. 34, 701.
Bonnet, V. (1934), Sur les coeurs lymphatiques de la grenouille et sur leur innervation. Journ. Physiol. et Pathol. Génér. 32, 745.
Borbola, J., Gy. Bikich & I. Faredin (1955), Histamine content of the ulcerous human gastric wall. Acta Med. Hung. 9, 163.
Borchard, A. (1928), Die primäre Lymphangitis des Wurmfortsatzes. Deutsche med. Wschr. 54, 1074.
Boros, J. & G. Czoniczer (1926), A vérplasma fehérjeszerkezetének az oedemával való összefüggéséről (Connection between the protein structure of the plasma and the formation of oedemas). Orv. Hetil. (Hungarian), 70, 318.
Borrmann, R. (1926), Geschwulste des Magens und Duodenums. In Hänke—Lubarsch, Handbuch der pathologischen Anatomie IV/1, 812.

Bellinazo, P. & H. Monteverde (1953), Minerva Chirurgica 8, 731. ref M. Martini (1954), Influence du système nerveux sympathique sur la circulation de la lymphe. Presse Méd. 62, 376.

Benczur, D. & A. Csatáry (1890), Über das Verhältnis der Ödeme zum Hämoglobin-Gehalt des Blutes. Deutsch Arch. klin. Med. 46, 478.

Benditt, E. P., S. Schiller, M. B Mathews & A Dorfman (1951), Evidence that hyaluronidase is not the factor in testicular extract causing increased vascular permeability. Proc. Soc. Exp Biol. Med. 77, 643.

Bennhold, H. (1938), Die Vehikelfunktion der Bluteiweißkorper. In Bennhold—Kylin—Rusznyák (1938), Die Eiweißkorper des Blutplasmas. Dresden u. Leipzig.

Bennhold, H (1951), Über die Bedeutung von physiologischen und von [illegible] erischem [illegible] plasma- [illegible] ır Frage [illegible] .p. Med 118, 407.

Bensley, S. H. (1934), On the presence, properties and distribution of the intercellular ground substance of loose connective tissue. Anat Record 60, 93.

Bensley, R. R. & B. Vimtrup (1928), On the nature of the Rouget cells Anat. Record. 39, 37.

Benson J A., P. R. Lee, J F Scholer, K. S. Kim & J. L. Bollman 1956, Water absorption from the intestine via portal and lymphatic pathways. Am J. Physiol. 184, 441.

Berde, B (1949), Über die Resorptionsgeschwindigkeit aus normaler und denervierter Haut. Dermatologica 98, 69

Bernard, C. (1857), Leçons sur les effets des substances toxiques et médicamenteuses. Paris.

Bert, P. & Laffont (1882), Influence du système nerveux sur les vaisseaux lymphatiques. C. R. Acad Sci 94, 739.

[illegible]

[illegible] bildes nach [illegible] Vergiftung. Deutsch. med. Wschr. [illegible]

Bierman, H. R., R. L Byron Jr, K H Kelly, R. S. Gilfilan, L P. White, [illegible] lymph, [illegible] Scand, Supp [illegible]

Biondi (1888), Beitrag zu der Struktur und Funktion der Schilddruse. Berl. klin Wschr. 25, 954.

Birke, G. (1953), The dermal spreading of hyaluronidase and streptococci under the influence of stress (cold) ACTH, cortisone and desoxycorticosterone acetate. Acta Med. Scand 144, 456.

Bizzozero, G. & G Salvioli (1878), Studii sulla struttura e sui linfatici delle sierose umane. Arch. per. le Sc. med. 2, 247

Blackburn, G., G. Hadfield & A. H. Hunt (1939), Regional ileitis St Barth Hosp. Ref. 72, 181.

Blalock, A. (1943), Study of thoracic duct lymph in experimental crush injury and injury produced by gross trauma Bull Johns Hopkins Hosp. 72, 54.

Blalock, A. & C S. Burwell (1935/36), Thoracic duct lymph pressure in concretio cordis. J. Lab Clin. Med. 21, 296.

Blalock, A., R. S. Cunningham & C. S. Robinson (1936), Experimental production of chylothorax by occlusion of the superior vena cava. An Surg. 104, 359.

Budge, A. (1880), Über ein Kanalsystem im Mesoderm von Hühnerembryonen. Arch. Anat. u Physiol. Anat. Abt. 320.

Budge, A. (1881), Über das dem zweiten Blutkreislaufe entsprechende Lymphgefäßsystem bei Hühnerembryonen Zbl. med Wissensch. 19, 625

*Budge, A. (1887), Untersuchungen über die Entwickelung des Lymphsystems* beim Hühnerembryo. Arch. Anat. u Physiol. Anat Abt. 59.

Bugár-Mészáros, K. (1944), Az érbetegségek diagnosztikája, kór- és gyógytana (Diagnosis, pathology and therapy of vessel diseases), MOKT (Hungarian), Budapest.

Bugha, G (1911), Untersuchungen über die Oberflächenspannung der Lymphe. Biochem. Z. 36, 411

Buno, W (1940), *Histologic verification of presence of colloid substance in* tributary lymphnode of thyroid, study of dissemenation of thyroid hormone Arch. Soc. Biol. Montevideo 10, 96.

Bunting, C. H. & J. Huston (1921), Fate of the lymphocyte. J. Exp Med 33, 593.

Burch, G, P. *Reaser & J Cronvich (1947), Rates of sodium turnover in* normal subjects and in patients with congestive heart failure J Lab. Clin. Med. 32, 1169.

Burch, G. E. & W A. Sodeman (1937), The estimation of the subcutaneous tissue pressure by a direct method. J. Clin Invest 16, 845

de Burgh, D. (1944), Some problems of lung physiology. Proc Cardiff. Med. Soc February, 12

*Burn, J H. & H H. Dale (1926/27), The vasodilator action of histamine and* its physiological significance J Physiol. 61, 185

Burrows, H. (1932), Some factors in the localization of disease in the body. Baillère, Tindall & Co, London.

Burton-Opitz, R & R Nemser (1918), The viscosity of lymph. Am J Physiol 45, 25.

Busey, S. C (1885), The causal relation of obstructed cardiac circulation to lymphstasis. Am, J Med Sci 90, 373.

Buxton, B. H. & J. C Torrey (1906/07), Absorption from the peritoneal cavity Part III The function of the diaphragm. J Med Research 15, 41.

*Cahen, R L & M Grasner (1944), Influence of morphine on tissue permea*bility and spreading effect of hyaluronidase Yale J Biol & Med 16, 257.

Cain, J. C., J H Grindlay, J. L Bollman, E V Flock & F C Mann (1947), Lymph from liver and thoracic duct. Surg Gynec & Obst 85, 558.

Calesnick, B & R Beutner (1949), Inhibition of hyaluronidase by aromatic *compounds. Proc Soc Exp Biol Med. 72, 629*

Callahan, W P, J C Sutherland, J K Fulton & J. R Kline (1952), Acute diffuse interstitial fibrosis of the lungs A M A Arch. Int. Med 90, 468.

Cameron, A T (1945), A textbook of biochemistry. 6. ed Churchill, London

Cameron, G R & F C. Courtice (1946), Production and removal of oedema fluid in lung after exposure to carbonylchloride (phosgene). J Physiol 105, 175.

Cameron, G R, F C. Courtice & R H. D Short (1947), Disturbances of function induced by lewisite and (2-chlorvinyldichlorarsine) Quart J. exp. Physiol 34, 1.

Cameron, G R & A H Sheikh (1951), The experimental production of pulmonary edema with ammonium salts, together with a classification of lung edemas. *J. Path Bact 63, 609.*

Camus, L (1894), Recherches sur les causes de la circulation lymphatique. Paris

Camus, L (1904), L'action de l'adrénaline sur l'écoulement de la lymphe C. R. Soc Biol. 56, 552

*Camus, L & E Gley (1894), Recherches expérimentelles sur les nerfs des* vaisseaux lymphatiques Arch physiol. norm et path. 26, 454.

Camus, L. & E Gley (1895a), Action du système nerveux sur les principaux lymphatiques C R. Acad Sci 120, 747.

Camus, L. & E Gley (1895b), Influence du sang asphyxique et de quelques poisons sur *la contractilité des vaisseaux lymphatiques. C. R. Acad Sci. 120, 1005.*

Bottazzi, F. (1908), Osmotischer Druck und elektrische Leitfähigkeit der Flussigkeiten der einzelligen, pflanzlichen und tierischen Organismen. Ergebn Physiol. 7, 161.

Bouveret (1893), Traité des maladies de l'estomac. Paris.

Bowman, F. B., M. C. Winternitz & H. M. Evans (1912), Über die vitale Färbung des Tuberkels. Zbl. Bakteriol. 65, 407.

Boyd, W. (1947), A Textbook of Pathology. 6. ed Lea and Febiger, Philadelphia.

(Braude, A I. *Брауде, А И.* (1948), О роли мезотелия во всасывании из брюшной полости. Бюлл эксп биол мед 24, 222..

(Braude, A. I.) *Брауде, А И.* (1950), Роль широких связок матки в образовании полостной жидкости Бюлл эксп биол мед 27, 143.

Braun, P , M Foldi, S Kisfaludy & Gy. Szabó (1956). Über den Gehalt der Lymphe an freien Aminosäuren Acta Med. Hung. 10, 67.

Braun, P., S. Kisfaludy & M. Dubsky (1955), Quantitative analysis of free aminoacids in normal and pathological human serum and urine Acta Med. Hung 7, 147.

Braun-Menendez E., Foglia V. G. (1910), Biologie et pharmacologie des coeurs lymphatiques des batraciens. Arch Internat Pharmacodyn et Therap. 64, 273

Braunsteiner, H , K. Fellinger & F. Pakesch (1953), Ergebnisse und Probleme histologischer Untersuchungen im Elektronmikroskop. Klin. Wschr. 31, 357.

Braus, H. (1940), Anatomie des Menschen. J. Springer, Berlin. IV. Band

Brendler, F. (1943), Übertritt von Kontrastmittel in das Lymphgefäßsystem bei der Hysterosalpingographie. Rontg. Prax. 11, 422

von der Brenle, H. A S. (1957), The effect of ionizing radiations on the regeneration and behaviour of mammalian lymphatics. Amer J. Roentgenol 78, 837.

Breslauer, F (1919), Die Pathogenese der tropischen Gewebsschäden nach der Nervenverletzung Deutsch Ztschr Chir 150, 50.

Brierley, J B. & E. J. Field (1948), The connexions of the spinal subarachnoid

il cases London

nogen in lymph.

upu minutového

154

es

Browicz (1900), Pathogenese des Ikterus Wiener Klin. Wschr 13, 785.

Brown, A L. (1937), Traumatic rupture of the thoracic duct with bilateral chylothorax and chylosus ascites. Arch Surg 34, 120

Brown, K. P. (1928), Peritoneal lymphatic absorption; experimental investigation to determine value of lymphaticostomy. Brit. J Surg 15, 538.

Brown, S & E. Hardenbergh (1950), A technique for sampling lymph in unanesthetized dogs by means of an exteriorized thoracic duct venous shunt. Naval Medical Research Institute (NM006 012 0431).

Bruce, A N. (1910), Über die Beziehungen der sensiblen Nervenendigungen zum Entzundungsvorgang Arch exp. Path. u Pharmakol. 63, 424.

Brucke (1853), Über die Chylusgefäße und die Resorption des Chylus. Sitzung Lymphherzens Frosches

Pflugers Arch 115, 334.

Buchanan, T. J., E. W. Walls & E. S. Williams (1954), Studies in radiosodium clearance from the skin in man. Clinical Science 13, 333

Buday, K. (1929), Kórboncolástan (Pathologic anatomy), Universitas (Hungarian), Budapest.

Budge, A. (1875), Neun Mitteilungen uber die Lymphgefäße der Leber Ber. Kgl. Sächs. Ges. Wiss, (Math -phys. Kl.) 161.

Budge, A. (1880), Über ein Kanalsystem im Mesoderm von Hühnerembryonen. Arch Anat. u. Physiol. Anat. Abt. 320.

Budge, A. (1881), Über das dem zweiten Blutkreislaufe entsprechende Lymphgefäßsystem bei Hühnerembryonen. Zbl med. Wissensch. 19, 625.

Budge, A. (1887), *Untersuchungen über die Entwickelung des Lymphsystems* beim Hühnerembryo Arch Anat u Physiol. Anat Abt. 59.

*Bugár-Mészáros, K (1944), Az érbetegségek diagnosztikája, kór- és gyógytana* (Diagnosis, pathology and therapy of vessel diseases). MOKT (Hungarian), Budapest.

Bugha, G (1911), Untersuchungen über die Oberflächenspannung der Lymphe. Biochem. Z. 36, 411.

Buno, W. (1940), Histologic verification of presence of colloid substance in tributary lymphnode of thyroid, study of dissemenation of thyroid hormone Arch. Soc Biol. Montevideo 10, 96.

Bunting, C. H & J. Huston (1921), Fate of the lymphocyte. J Exp Med. 33, 593.

Burch, G, P. Reaver & J Cronvich (1947), Rates of sodium turnover in normal subjects and in patients with congestive heart failure. J. Lab. Clin. Med. 32, 1169

Burch, G E. & W. A Sodeman (1937), The estimation of the subcutaneous tissue pressure by a direct method J. Clin. Invest 16, 845.

de Burgh, D. (1944), Some problems of lung physiology Proc Gardiff Med. Soc February, 12

Burn, J. H. & H H. Dale (1926/27), The vasodilator action of histamine and its physiological significance. J. Physiol 61, 185

Burrows, H (1932), Some factors in the localization of disease in the body Ballière, Tindall & Co., London.

Burton-Opitz, R & H *Nemser (1918), The viscosity of lymph* Am. J Physiol. 45, 25.

Busey, S C *(1885), The causal relation of obstructed cardiac circulation to* lymphstasis Am, J Med. Sci. 90, 373.

Buxton, B H & J C *Torrey (1906/07), Absorption from the peritoneal cavity*. Part III The function of the diaphragm J Med Research 15, 41

*Cahen, R. L & M. Grainer (1944), Influence of morphine on tissue permeability* and spreading effect of hyaluronidase Yale J. Biol & Med 16, 257.

*Cain, J C, J H. Grindlay, J L Bollman, E V. Flock & F. C Mann (1947)*, Lymph from liver and thoracic duct Surg Gynec & Obst 85, 558.

*Calesnick, B & R* Beutner (1949), *Inhibition of hyaluronidase by aromatic* compounds. Proc Soc Exp Biol Med 72, 629

*Callahan, W P, J C Sutherland, J K Fulton & J R Kline (1952), Acute* diffuse interstitial fibrosis of the lungs A M A Arch Int Med. 90, 468

*Cameron, A T (1945), A textbook of biochemistry 6 ed.* Churchill, London.

Cameron, G R & F C Courtice (1946), Production and removal of oedema *fluid in lung after exposure to carbonylchloride (phosgene)* J. Physiol. 105, 175.

Cameron, G R, F C Courtice & R H D Short (1947), Disturbances of function *induced by lewisite and (2-chlorvinyldichlorarsine) Quart. J. exp* Physiol. 34, 1.

Cameron, G R & A H Sheikh (1951), The experimental production of pul*monary edema with ammonium salts, together with a classification of lung edemas.* J. Path Bact 63, 609

*Camus, L. (1894), Recherches sur les causes de la circulation lymphatique.* Paris

Camus, L (1904), L'action de l'adrénaline *sur l'écoulement de la lymphe*. C. R. Soc Biol 56, 552

*Camus, L. & E Gley (1894), Recherches expérimentelles sur les nerfs des* vaisseaux lymphatiques Arch physiol norm et path 26, 454.

*Camus, L & E Gley (1895a), Action du système nerveux sur les principaux* lymphatiques C. R Acad Sci 120, 747

Camus, L. & E. Gley (1895b), *Influence du sang asphyxique et de quelques* poisons sur la contractilité des vaisseaux lymphatiques C. R. Acad. Sci. 120, 1005.

Camus, L. & E Gley (1895 c), Recherches sur l'innervation du canal thoracique. Arch. physiol norm. et path. 7, 301.

Cannon, W. B. (1923), Traumatic shock. D. Appleton & Co., New York.

Carlson, A. J., J. R. Greer & F. C. Becht (1907), On the mechanism by which water is eliminated from the blood in the active salivary gland. Am. J. Physiol 19, 360.

Carlsten, A. (1950), On the sources of the histamine present in thoracic duct [illegible]

[illegible] ic [illegible] ph [illegible] J. Urol. 26, 105

Carrier, E B. & P. B. Rehberg (1923), Capillary and venous pressure in man Skand. Arch. Physiol. 44, 20

Caylor, H. D., C. F. Schlotthover & J. Pemberton (1927), Observations on the lymphatic connections of the thyroid gland. Anat Record. 36, 325

Chaikoff, I L , B. Bloom, B. P. Stevens, W. O. Reinhardt & W. G. Dauben (1951), Pentadecanoic acid — 5—$C^{14}$ Its absorption and lymphatic transport. J Biol. Chem. 190, 431.

Chaikoff, I. L., B. Bloom, M. D. Siperstein, I. J. Kiyasa, W G Reinhardt, W. G Dauben & J F. Eastham (1952), $C^{14}$ cholesterol. I Lymphatic transport of an absorbed cholesterol — 4—$C^{14}$ J. Biol. Chem 194, 407

Chain, E. & E S Duthie (1940), Identity of hyaluronidase and spreading factor Brit J. Exp Path. 21, 324.

Chambers, R. & B. W. Zweifach (1940), Capillary endothelial cement in relation to permeability. J. Cell Comp. Physiol. 15, 255

Chambers, R & B W. Zweifach (1944), Topography and function of the mesenteric capillary circulation Am. J. Anat. 75, 173.

Chambers, R & B. W. Zweifach (1947), Intracellular cement and capillary permeability. Physiol. Rev 27, 436.

Charrié (1896), Action des toxines sur la lymphe. Gaz. méd. Paris 367

Cheng, K (1949), Observations on dye excretion through synovial membrane after lumbosacral sympathectomy and circulatory obstruction. Quart J. Exp. Physiol. 35, 135.

Chesney, A. M., C. B Turner & C. R. L. Halley (1928), Studies in experimental syphilis VIII. On the localisation of syphilitic lesions in inflammated areas Bull. Johns Hopkins Hosp. 42, 319.

Chiari, R. & H. Januschke (1910), Hemmung von Transsudat- und Exsudatbildung durch Calciumsalze Wien Klin. Wschr. 23, 427.

Child, C. G. (1954), The hepatic circulation and portal hypertension. Saunders, Philadelphia—London.

Chinard, F P., H. D. Lauson, H. A. Eder, R L Greif & R. Hiller (1954), A study of the mechanism of proteinuria in patients with the nephritic syndrome. J. Clin. Investigation. 33, 621

Chittenden, R. H., L. B. Mendel & J Henderson (1899), A chemico-physiological study of certain derivates of proteids. Am J. Physiol, 2, 142.

Christoni, A. (1921), Contributo allo studio della linfogenesi. Arch. di fisiol. 19, 101

Churchill, E D , F. Nakazawa & C. K. Drinker (1927), The circulation of body fluids in the frog. J. Physiol. 63, 304.

Clark, E. R. (1909), Observations on living growing lymphatics in the tail of the frog larva. Anat. Record. 3, 183.

Clark, E. R. (1936), Growth and development of function in blood vessels and lymphatics Ann. Int. Med 9, 1043.

[illegible] rbon and carmine [illegible] phatics in experimental [illegible]

Clark, E. R. & E. L. Clark (1925), The relation of Rouget's cells to capillary contraction. Am. J. Anat. 35, 265.

Clark, E. R. & E L. Clark (1932), Observations on new growth of lymphatic vessels as seen in transparent chambers introduced into rabbit's ear. Am. J. Anat. 51, 49.

Clark, E. R. & E. L. Clark (1933), Further observations on living lymphatic vessels in the transparent chamber in the rabbit's ear — their relation to tissue spaces. Am J. Anat. 52, 273.

Med 62, 229.

Claude, A. & F. Duran-Reynals (1934), On existence of factor-increasing tissue permeability in organs other than testicle J Exp Med. 60, 457.

Claude, A. & F. Duran-Reynals (1937), Chemical properties of the purified spreading factor from testicles. J. Exper. Med 65, 661.

Cohnheim, J. (1877/80), Vorlesungen uber allgemeine Pathologie. A Hirschwald, Berlin.

Cohnheim, J. & L. Lichtheim (1877), Ueber Hydraemia und hydraemisches Oedem. Virchows Arch. 69, 106.

Cohnstein, W (1895a), Ueber Resorption aus der Peritonealhoble. Centralbl. Physiol 9, 401.

Cohnstein, W (1895b), Weitere Beiträge zur Lehre von der Transsudation und zur Theorie der Lymphbildung Pflugers Arch. 59, 350.

Cohnstein, W (1895c), Ueber die Einwirkung intravenoser Kochsalzinfusionen auf die Zusammensetzung von Blut und Lymphe. Dritter Beitrag zur Theorie

, 508

zu meiner im 59. Bande dieses Archivs

nwirkung intravenoser Kochsalzinfusionen

d Lymphe« Pflugers Arch 60, 291.

intravenose Infusionen hyperisotonischer

r Lymphbildung) Pflügers Arch 62, 58

Hydrops Erg Allg Path u. path Anat

3, 563

Cohnstein, W. (1896b), Ueber die Theorie der Lymphbildung (6 Mitteilung.) Pflugers Arch 63, 587.

cortisone on serum non-specific

Proc. Exp Biol, Med. 77, 363

Wound healing, cutaneous and

study. Surg. Gynec & Obst

90, 269.

Colin, G (1871/73), Traité de physiologie comparée des animaux. Ballière et Fils 2 éd Paris

Colin, G (1879), Recherches expérimentales sur le mode de développement des infiltrations et des hydropsies passives Bull. Acad Méd 8, 1283

Colson (1890), Recherches physiologiques sur l'occlusion de l'aorte thoracique. Arch de Biol 10, 431

Comparini, L. (1958), La minuta struttura dei collettori linfatici superficiali e profondi degli arti nell'uomo. Siena, Tipografia Nouva

Condorelli, L (1931), Die Bedeutung der venosen Zirrhose fur die Pathogenese des Vorhofflimmerns. Verhandl deutsch Gesellsch. Kreislaufforschung. 225.

Condorelli, L (1932), Die Ernährung des Herzens und die Folgen ihrer Storung. Steinkopff, Dresden

Conklin, R S (1930), The formation and circulation of lymph in the frog. I The rate of lymph production. Am J Physiol. 95, 79.

Conklin, R. S. (1930), The formation and circulation of lymph in the frog II Blood volume and pressure. A. J. Physiol. 95, 91.

Conklin, R. S. (1930), The formation and circulation of lymph in the frog III. The permeability of the capillaries to protein. Am. J. Physiol. 95, 98

Cope, O., A. G. Brenizer Jr. & H. Polderman (1942), Capillary permeability and the adrenal cortex; studies of cervical lymph in the adrenalectomized dog Am J Physiol. 137, 69.

Cope, O. & F. D. Moore (1944), A study of capillary permeability in experimental burns and burn shock using radio-active dyes in blood and lymph J. Clin Invest 23, 241.

Cope, O, J. B Graham, G. Mixter & M. R. Ball (1949), Threshold of thermal trauma and influence of adrenal cortical and posterior pituitary extracts on the capillary and chemical changes Arch. Surgery. 59, 1015.

Corcoran, A. C. & I. H. Page (1943), Effects of hypertension due to hemorrhage and [illegible] ed. 67, 205.

[illegible] effects on renal func[illegible] urniquets. Ann. Surgery. 118, 871.

Cordier, G & Nguyen Huu (1952), Les gaines vasculaires des pédicules pulmonaires, leur intérêt anatomo-chirurgical. J. fr méd. chir. thorac. 6, 409.

Co Tui, I. S. Barcham & B G. P. Shafiroff (1949), Ligation of the thoracic [illegible] Surg. Gynec. & Obst. 89, 37.

[illegible] boratory [illegible] .l of free red blood cells from the peritoneal cavity of animals. Australian J. Exp Biol & Med. Sci. 31, 215.

Courtice, F. C. & P I. Korner (1952), The effect of anoxia on pulmonary edema produced by massive intravenous infusions. Austral. J. Exp. Biol & Med. Sci. 30, 511.

Courtice, F. C. & B Morris (1953), The effect of diaphragmatic movement on the absorption of protein and of red cells from the pleural cavity Austral J. Exp Biol & Med. Sci 31, 227.

Courtice, F C. & P. J. Phipps (1946), The absorption of fluids from the lung J. Physiol 105, 186.

Courtice, F. C. & W. J. Simmonds (1949a), Absorption from the lungs J. Physiol. 109, 103.

Courtice, F. C. & W. J Simmonds (1949b), Absorption of fluids from the pleural cavities of rabbits and cats. J. Physiol 109, 117.

[illegible] of protein from the [illegible] mph [illegible] isma [illegible] ruc-tion and of posture on the absorption of protein from the peritoneal cavity. Austral. J. Exp. Biol & Med Sci. 29, 451.

Courtice, F. C & A. W. Steinbeck (1951b), Absorption of protein from peritoneal cavity. J Physiol. 114, 336.

Cradock, C. G, W. N. Valentine & J S. Lawrence (1949), The lymphocyte. Studies on its relationship to immunologic processes in the cat J. Lab. Clin Med. 34, 158

Cressman, R D. & A Blalock (1939), The effect of pulse upon the flow of lymph. Proc. Soc. Exp. Biol. Med 41, 140.

Crooke, A. D. & C J. O. Morris (1942), Determination of plasma volume by Evans blue method. J. Physiol 101, 217.

Cruikshank, W. (1789), Geschichte und Beschreibung der einsaugenden Gefäße oder Saugadern des menschlichen Korpers. Leipzig.

Cruikshank, V. (1790), The anatomy of the absorbing vessels of the human body. London.

Cunningham, R. S. (1922a), Studies in absorption from serous cavities. IV. On the passage of blood cells and granules of different sizes through the walls of the lymphatics in the diaphragm. Am. J. Physiol. 62, 248.

Cunningham, R. S. (1922b), Studies in absorption from serous cavities. V. The absorption of particulate matter from the peritoneal cavity of the fetus. Am. J Physiol. 62, 253.

Cunningham, R. S (1926), The physiology of the serous membranes. Physiol. Reviews 6, 242.

Cunningham, R. S. (1927), Studies on absorption from serous cavities. VI. The effect of ligation of the mediastinal lymphatics on the absorption from the peritoneal cavity. Am. J. Physiol. 24, 129.

Cushny, A. R. (1926), The secretion of the urine. Longmans, Green & Co., London.

Dabelow, A (1930/31), Reaktionsweisen des Lymphknotens beim Fetttransport (unter besonderer Berücksichtigung des Mesenterialknotens). Z Zellforsch. u mikr Anat. 12, 207.

Dähnhardt, C. (1866), Zur Chemie der Lymphe. Virchows Arch. 37, 55.

Dale, H. H. (1929), Croonian lectures on some chemical factors in control of circulation. Lancet 1, 1179

Dale, H. H. (1935), Discussion on traumatic shock. Proc. Roy. Soc. Med 28, 1493.

Dale, H. H. & P. P. Laidlaw (1911/12), Further observations on the action of β-iminazolethylamine. J. Physiol 43, 182.

Dale, H. H. & P. P. [illegible] of short-circuiting the portal circulation. J. [illegible]

Dale, H. H. & P. P. [illegible] Physiol 52, 355.

Dale, H. H. & A. N [illegible] action of histamine and of some other substa[illegible]

Dalmady, Z. (1911), Kísérletek a bőr nyirokkeringésének klinikai vizsgálatára (Experiments concerning the clinical examination of the lymph circulation of the skin) Orv. Hetilap (Hungarian), 36.

Dandy, W. (1929), Where is the cerebrospinal fluid absorbed? J. A. M A. 92, 2012.

Danielli, J F (1940), Capillary permeability and oedema in perfused frog J. Physiol 98, 109

[illegible]

Davis, B. F. & A J Carlson (1909/10), Contribution to the physiology of lymph. IX. Notes on the leukocytes in the neck lymph, thoracic lymph and blood of normal dogs. Am J Physiol 25, 173

Davis, J. O, A E Lindsay & J L Southworth (1952), Mechanisms of fluid and electrolyte retention in experimental preparations in dogs. Bull. Johns Hopkins Hosp 90, 64

Day, T (1948), The spread of fluids in connective tissue. J. Pathol. & Bacter. 60, 150

Day, T D. (1949), The mode of reaction of interstitial connective tissue with water J Physiol 109, 380

Day, T (1952), The permeability of interstitial connective tissue and the nature of the interfibrillary substance. J Physiol. 117, 1.

Day, T & G. Eaves (1953), Electron microscope observations on the ground substance of interstitial connective tissue. Biochim & Biophys. Acta. 10, 203.

Delarue, J, R. Depierre & J. Roujeau (1950), Lymphangiectasie pulmonaire et pneumonie chyleuse. Sem Hôp. Paris. 26, 4906.

Dencker, J. & I. Gottfries (1954), Cortisone in the treatment of chronic hereditary oedema. Acta. Med. Scand. **150**, 277.

Denecke, G. (1914), Ueber die Bedeutung der Leber fur die anaphylaktische Reaktion beim Hunde. Z. Immunforsch. **20**, 501.

Dexter, L (1949), Pulmonary circulatory dynamics in health and disease, at rest. Bull. New England Med. Center. **11**, 240.

Dexter, L., J. W. Dow, F. W. Haynes, J. L. Whittenberger, B. G. Ferris, W. T. Godah & H. K. Hellems (1950), Studies of pulmonary circulation in man at rest Normal variation and interrelations between increased pulmonary blood flow, elevated pulmonary arterial pressure and high pulmonary "capillary" pressures J. Clin. Investig. **29**, 602.

Deyrup, I. J. (1944), Circulatory changes following subcutaneous injection of histamine in dogs. Am. J. Physiol. **142**, 158.

Dible, J H. & G. A. C. Lynch (1938), Chronic and fatal form of hydrothorax produced in rabbits by intrapericardial injection of tincture of iodine and mechanism of its production J. Path. & Bact. **46**, 271.

Diczfalusy, E., E. Ferno, H. Fex, B. Hogberg, T. Linderot & Th Rosenberg (1953), Synthetic high molecular enzyme inhibitors. I. Polymeric phosphates of phloretin and related compounds. Acta Chem. Scand. **7**, 913.

de Diemerboeck, I. (1685), Opera Omnia Anatomica et medica Dreunen, Utrecht

Dietrich, A. (1924), Gewebsquellung und Ödem in morphologischer Betrachtung. Virchows Arch. **251**, 533.

Disse, J. (1890), Über die Lymphbahnen der Saugetierlunge. Arch. mikr Anat. **36**, 203

Disse, J. (1911), Die Lymphbahnen der menschlichen Magenschleimhaut. Arch. f. mikr. Anat. **78**, 74

Dobyns, B. M. & E. Z. Hirsch (1956), Iodinated compounds in the lymphatic pathways leading from the thyroid J. Clin. Endocrin. a. Metabol. **16**, 153.

Dogiel, A. (1880), Über ein, die Lymphgefaße umspinnendes Netz von Blutkapillaren. Arch mikr. Anat. **17**, 335.

Dogiel, A. (1883), Über die Beziehungen zwischen Blut- und Lymphgefaßen Arch. mikr. Anat. **22**, 608.

Dogiel, A. (1897), Die Nerven der Lymphgefaße Arch. mikr. Anat **49**, 791.

Dole, V. P., K Emerson, R A. Phillips, P. Hamilton & D D Van Slyke (1945/46), The renal extraction of oxygen in experimental shock. Am J. Physiol **145**, 337.

Doljanski, L & Fr. Roulet (1933), Studien uber die Entstehung der Bindegewebsfibrille. Virch Arch **291**, 260.

Dolley, F. S. & E. R Wiese (1929), Effects of a large closed bilateral pneumothorax on thoracic lymph flow. Arch. Surgery. **18**, 542

Dorfman, A & M. L Ott (1948), A turbidimetric method for assay of hyaluronidase. J. Biol Chem. **172**, 367.

Dorfman, A., M L. Ott & R Whitney (1948), The hyaluronidase inhibitor of human blood J. Biol. Chem. **174**, 621.

Dorfman, A., E. J. Reimers & M. L. Ott (1947), Action of sodium salicylate on hyaluronidase Proc. Soc. Exper. Biol. Med **64**, 357.

Dougherty, T. F. & A White (1944), Influence of hormones on lymphoid tissue structure and function. The role of the pituitary adrenocorticotropic hormone in the regulation of the lymphocytes of the blood. Endocrinology **35**, 1.

Dougherty, T. F & A. White (1945), Functional alterations in lymphoid tissue induced by adrenal cortical secretion Am. J. Anat. **77**, 81.

Dougherty, T F. & A White (1946), Pituitary adrenal cortical control of [illegible] by experimental X-ray radiation.

[illegible] valuation of alteration produced in lymphoid tissues by pituitary adrenal cortical secretion. J. Lab. & Clin. Med. **32**, 584

Dougherty, T. F. & L. F. Kumagai (1951), Influence of stress stimuli on lymphatic tissue of adrenalectomized mice. Endocrinology **48**, 691.

Doyle, J. T., J. S. Willson, E. Hr. Estes Jr. & J. V. Warren (1951), Effect of intravenous infusion of physiological saline solution on pulmonary arterial and pulmonary capillary pressure in man. J Clin Investig. 30, 345.

Doyon, R. (1903), Action de la peptone sur la sécrétion et l'excrétion de la bile. C. R. Soc. Biol. 55, 314.

Drake, T. G. H & F. F. Tisdal (1926). The effect of histamine on the blood chlorides J. Biol. Chem. 67, 91.

Drennan, J. M. (1951), Mast cells in urticaria pigmentosa. J. Path. & Bact. 63, 513.

Drinker, C. K. (1927), The permeability and diameter of the capillaries in the web of the brown frog (R. Temporaria) when perfused with solutions containing pituitary extract and horse serum. J. Physiol. 63, 249

Drinker, C. K. (1938), The functional significance of the lymphatic system. Bull. New York Acad. Med. 14, 231

Drinker, C. K. (1945), Pulmonary edema and inflammation Harward Univ. Press, Cambridge Mass.

Drinker, C. K. (1946), Extravascular protein and lymphatic system Ann. New York. Acad. Sc. 46, 807.

Drinker, C. K. & M E. Field (1931), Absorption from the pericardial cavity. J. Exp Med. 53, 143

Drinker, C K. & M. E. Field (1933), Lymphatics, lymph and tissue fluid. Williams and Wilkins, Baltimore.

[illegible] iction [illegible] 509

[illegible] mpo- [illegible] from [illegible]

[illegible]

tio [illegible] an [illegible] of [illegible]

Drinker, C. K, M D. Warren & M. Mac-Lanahan (1938), The absorption of protein solutions from the pulmonary alveoli. J. Exp. Med 66, 449.

Drinker, C. K., M. D. Warren, F. V Maurer & J D McCarrell (1940), The flow, pressure and composition of cardiac lymph. Am. J. Physiol 130, 43.

Drinker, C K. & J. M Yoffey (1941), Lymphatics, lymph and lymphoid tissue Harward Univ. Press Cambridge Mass.

Drinker, C K & M. F Warren (1943), Genesis and resolution of pulmonary transudates and exsudates (Alpha Omega Alpha lecture). J A M A. 122, 269.

Drinker, C K & E Hardenbergh (1947), Absorption from pulmonary alveoli. J. Exp. Med 86, 7

(Drozdova, A V) *Дроздова, А В* (1953), Коллатеральное лимфообращение тонком кишечнике в условиях неполной десимпатизации и деафферентации. Арх анат. гист и эмбриол 30/4, 63

Drukman, A & S Rozin (1951), Uterovenous and uterolymphatic intravasation [illegible]

D[illegible] ich oedema forms [illegible]

D[illegible] sécrétoire Arch Anat Microsc 15, 53.

Dudrey, J (1899), Les voies sanguines et lymphatiques dans l'absorption péritonéale C R Soc. Biol 84, 172

Duncan, G W. & A Blalock (1942 a), Shock produced by crush injury, effects of administration of plasma and local application of cold Arch. Surgery 45, 183.

Duncan, G W & A Blalock (1942 b), Uniform production of experimental shock by crush injury; possible relationship to clinical crush syndrome. Ann. Surg. 115, 684.

Duran-Reynals, F. (1928), Exaltation de l'activité du virus vaccinal par les extraits de certains organes C. R Soc Biol 99, 6

Dencker, J. & I Gottfries (1954), Cortisone in the treatment of chronic hereditary oedema. Acta. Med. Scand. 150, 277.

Denecke, G. (1914), Ueber die Bedeutung der Leber fur die anaphylaktische Reaktion beim Hunde. Z Immunforsch. 20, 501.

Dexter, L (1949), Pulmonary circulatory dynamics in health and disease, at rest. Bull. New England Med. Center. 11, 240.

Dexter, L., J. W. Dow, F. W Haynes, J. L. Whittenberger, B. G. Ferris, W. T. Godah & H. K Hellems (1950), Studies of pulmonary circulation in man at rest. Normal variation and interrelations between increased pulmonary blood flow, elevated pulmonary arterial pressure and high pulmonary "capillary" pressures. J. Clin Investig 29, 602.

Deyrup, I. J. (1944), Circulatory changes following subcutaneous injection of histamine in dogs Am. J. Physiol 142, 158.

Dible, J. H & C. A. C. Lynch (1938), Chronic and fatal form of hydrothorax produced in rabbits by intrapericardial injection of tincture of iodine and mechanism of its production J. Path. & Bact. 46, 271

Diczfalussy, E , E. Ferno, H Fex, B. Hogberg, T. Linderot & Th. Rosenberg (1953), Synthetic high molecular enzyme inhibitors. I. Polymeric phosphates of phloretin and related compounds Acta Chem Scand 7, 913.

de Diemerboeck, I. (1685), Opera Omnia Anatomica et medica Dreunen, Utrecht.

Dietrich, A (1924), Gewebsquellung und Ödem in morphologischer Betrachtung. Virchows Arch 251, 533

Disse, J (1890), Über die Lymphbahnen der Saugetierlunge Arch. mikr. Anat. 36, 203

Disse, J. (1911), Die Lymphbahnen der menschlichen Magenschleimhaut Arch. f. mikr. Anat. 78, 74

Dobyns, B M. & E Z. Hirsch (1956), Iodinated compounds in the lymphatic pathways leading from the thyroid. J Clin. Endocrin. a Metabol 16, 153

Dogiel, A (1880), Über ein, die Lymphgefaße umspinnendes Netz von Blutkapillaren Arch mikr. Anat. 17, 335.

Dogiel, A (1883), Über die Beziehungen zwischen Blut- und Lymphgefaßen. Arch mikr. Anat 22, 608.

Dogiel, A. (1897), Die Nerven der Lymphgefaße Arch. mikr. Anat. 49, 791

Dole, V P , K Emerson, R. A Phillips, P Hamilton & D. D. Van Slyke (1945/46), The renal extraction of oxygen in experimental shock. Am J. Physiol 145, 337

Doljanski, L & Fr Roulet (1933), Studien uber die Entstehung der Bindegewebsfibrille. Virch. Arch. 291, 260.

Dolley, F S. & E. R. Wiese (1929), Effects of a large closed bilateral pneumothorax on thoracic lymph flow. Arch Surgery. 18, 542.

Dorfman, A & M. L Ott (1948), A turbidimetric method for assay of hyaluronidase J. Biol Chem 172, 367

Dorfman, A., M L Ott & R Whitney (1948), The hyaluronidase inhibitor of human blood. J. Biol Chem 174, 621.

Dorfman, A , E. J. Reimers & M. L. Ott (1947), Action of sodium salicylate on hyaluronidase Proc. Soc Exper. Biol Med 64, 357.

Dougherty, T. F. & A. White (1944), Influence of hormones on lymphoid tissue structure and function The role of the pituitary adrenocorticotropic hormone in the regulation of the lymphocytes of the blood. Endocrinology. 35, 1

Dougherty, T F. & A White (1945), Functional alterations in lymphoid tissue induced by adrenal cortical secretion. Am. J. Anat. 77, 81.

Dougherty, T. F. & A White (1946), Pituitary adrenal cortical control of lymphocyte structure and function as revealed by experimental X-ray radiation. Endocrinology 39, 370.

Dougherty [illegible] ed in [illegible] 584.
[illegible] di on [illegible]

Elster, S K., M. E. Freeman & P. R. Anderson (1949), The effect of hyaluronidase on the hematocrit and plasma proteins of the albino rat. J. Lab. & Clin. Med. 34, 834

Elster, S. K, M. E. Freeman & M. Dorfman (1949), Effect of hyaluronidase on passage of fluid and of T-1824 through capillary wall Am. J. Physiol 156, 429.

Eltrich, T. & C. C. Yount (1925), Congenital elephantiasis Amer. J Dis. Child 29, 59

der Lymphabsonderung vom

T Ness, F. A. Clarke & E. R. Adamık (1949), A study of histochemical iron using tracer methods. J Lab & Clin Med 34, 414.

Engel, D (1941), The influence of the sympathetic nervous system on capillary permeability J Physiol 99, 161.

Engels, W (1904), Die Bedeutung der Gewebe als Wasserdepots. Arch exp Path 51, 346.

Eöllős, Z., I Rusznyák, M Földı & Gy Szabó (1949), Hyaluronıdase hatása fehérje és baktériumok nyirokerek útján történő felszívódására (Effect of hyaluronidase on the absorption of protein and bacteria through lymph vessels) Orv Hetil. (Hungarian), 90, 711

Eppinger, H (1902), Beiträge zur normalen und pathologischen Histologie der menschlichen Gallenkapillaren mit bes Berücksichtigung der Pathologie des Ikterus (Auf Grund einer neuen Färbungsmethode) Zieglers Beitr. 31, 230

Eppinger, H. (1903), Weitere Beiträge zur Pathologie des Ikterus Zieglers Beitr. 33, 123

Eppinger, H (1913), Ueber eine eigentumliche Hautreaktion hervorgerufen durch Ergamin. Wien. med Wschr 63, 1413.

Eppinger, H. (1920), Die hepatolienalen Erkrankungen Berlin

Eppinger, H (1935), Zum Problem der serosen Entzündung. Wien Klin Wschr. 48, 665.

Wien.

Entzündung, eine

analoge Zustände

ng des Lymphapparates

s Gewebe sowie das Lym 43

Genitales. Frschr Ro-Str 80, 627

rastmittel in das Lymph-Str 80, 633.

silicatosisa (Silicosis of ngarian]), 5, 44

Acut diffus interstitialis ), Magyar Belorv. Arch. (Hungarian), 7, 125

D'Errico, G (1906/07), Contributo allo studio delle vie di assorbimento del grasso alimentare. Arch di fiziol. 4, 513.

Esmarch, F & D Kuhlenkampff (1885), Die elephantiatischen Formen Richter, Hamburg

Duran-Reynals, F. (1942), Tissue permeability and spreading factors in infection: contribution to host-parasite problems. Bact. Rev 6, 197.

Duran-Reynals, F. (1952), La substance fondamentale du mésenchyme et les facteurs de diffusion dans l'infection. Sem. Hôp. Paris 28, 1047.

Duran-Reynals, F. (1954), Some remarks on the spreading reaction, in Asboe-Hansen.

*Duranteau, M.*, F Oury, C. Proux & L. Leger (1955), Essais de lymphographie abdomino-thoracique par injection intrapéritonéale de substances iodées. Presse Médicale 63, 1586

Durr, R. (1924), Bantimilz und hepatolienale Fibrose. Zieglers Beitr 72, 418.

(*Dvizhkov, P. P.*) *Движков, П. П* (1951), Изменения лимфатической системы при силикозе. Силикоз труды АМН СССР, XVII, 121, Медгиз, Москва

Dybkovski (1866), Ueber Aufsaugung und Absonderung der Pleurawand Arch. Physiol. Anst. Leipzig 1, 60.

Ebbecke, U. (1917), Die lokale vasomotorische Reaktion (L. V R ) der Haut und der inneren Organe Pflugers Arch 169, 1.

Eberth & A. Belajeff (1866), Über die Lymphgefäße des Herzens Virchows Arch. 37, 124

Ebner (1896), Die Chorda dorsalis der niederen Fische und die Entwickelung des fibrillären Bindegewebes Z Zool. 62, 469

Ebner, V (1899), Vom Gefäßsystem. Koelliker's Handbuch der Geweblehre des Menschen. Leipzig

Ecker, A (1846), Der feinere Bau der Nebennieren beim Menschen und den vier Wirbelthierklassen Braunschweig

Eckstein, H. C. (1925), Fat absorption through channels others than the left thoracic duct. J. Biol Chem 62, 737.

Eder, H., C V Ha- ... fibrosis of the lungs. B ...

Edgerley, R. H (1 ... substance in mice. Am ...

Edgerley, R. H (1953), Effect of X-irradiation on connective tissue ground substance. Am. J. Physiol. 174, 341.

Ehrenhaft, J. L & R Meyers (1948), Blood fat levels following supradiaphragmatic ligation of the thoracic duct Ann Surgery. 128, 38.

Ehrich, W. E (1945/46), The role of the lymphocyte in the circulation of the lymph. Ann. New York Acad. Sci. 46, 823.

Ehrich, W. E., J Seifter, H E. Alburn & A J. Begamy (1949), Heparin and ... Proc. Soc. Exp. Biol. Med. 70, 183

... Kenntnis der granulierten Bindegewebszellen ... rch Anat. u Physiol, Physiol Abt, Suppl ... 100.

Eickhoff W., J Kracht & W. Horst (1956), Untersuchungen über den Hormonjodtransport im Lymphsystem des Halses. Verhandl d deutsch. Gesellsch f. Pathologie, 40. Tagung, Dusseldorf.

Eisenmenger, W. J. & R J. Slater (1953), Distribution and decay of I[131] tagged albumin and gamma-globulin in patients with cirrhosis J. Clin Investig 32, 564.

Ekehorn, G. (1931), On the principles of renal function. Acta Med Scand. Suppl. 361.

Elford, W. J & J D. Ferry (1935), The calibration of graded collodion membranes Brit. J. Exp. Path 16, 1.

Elias, H (1953), Functional morphology of the liver. Research in the Service of Medicine 37, 26

Ellinger, A (1902), Die Bildung der Lymphe. Ergebn. Physiologie I. Abt. 1, 355.

Ferrar, M., N. L. Caubarrere, M. D. Bottinelli, C. Mendilaharsa & D. Giudice (1949), Neumofibrosis intersticial evolutiva, Una nueva entidad? Hoja tisiol. 9, 207, ref: Am. Rev. Tuberc 61, 89 (1950)

Ferry, J. D. (1936a), Statistical evaluation of sieve constants in ultrafiltration. J. Gen. Physiol. 20, 95.

Ferry, J. D. (1936b), Ultrafilter membranes and ultrafiltration Chem. Rev. 28, 373.

Field, E. J. & J. B. Brierley (1948), The lymphatic drainage of the spinal nerve in the rabbit. J. Anat. 82, 198.

Field, M. E. (1935), The reactions of the blood capillaries of the frog and rat to mechanical and electrical stimulation. Skand. Arch Physiol. 72, 175

Field, M. E & C. K. Drinker (1931a), Permeability of capillaries of dog to protein. Am. J. Physiol. 97, 40.

Field, M. E. & C. K. Drinker (1931b), Conditions governing the removal of protein deposited in the subcutaneous tissue of the dog. Am. J. Physiol. 98, 66

Field, M. E. & C K. Drinker (1931c), The rapidity of interchanges between [illegible]

[illegible] the dog. Am. J. Physiol 110, 174.

Field, M. E , M. F. Shaffer, J. F. Enders & C. K. Drinker (1937), The distribution in the blood and lymph of pneumococcus type III injected intravenously in rabbits, and the effect of treatment with specific antiserum on the infection of the lymph. J Exp. Med. 65, 469.

Field, M E. & J B Brierley (1949), Retro-orbital tissues as site of outflow of cerebrospinal fluid Proc. Roy Soc Med. 42, 477

Field, M. E., O C. Leigh Jr., J. W. Heim & C K. Drinker (1934/35), The protein content and osmotic pressure of blood serum and lymph from various sources in the dog Am J Physiol 110, 174

Fine, J. & A. M Seligman (1943a), Traumatic shock IV. A study of the problem of the "lost plasma" in haemorrhagic shock by the use of radio-active plasma protein J. Clin. Investig. 22, 285

[illegible] tal study including evidence

a [illegible] ry 118, 238

[illegible] VII A study of the problem

o [illegible] burn shock by the use of radio-active iodoplasma protein J Clin. Investig. 23, 720.

Fink, R M , T. Enns, C P Kimball, H. E. Silberstein, W. F. Bale, S C Madden & G. P Whipple (1944), Plasma protein metabolism-normal and associated with shock, observations using protein labeled by heavy nitrogen in lysine. J. Exp. Med. 80, 455

Fischer, E (1933a), Eine einfache Methode zur Darstellung der Lymphgefäße durch parenchymatose Injektion von Luft Arch klin. Chir. 176, 17.

Fischer, E. (1933b), Untersuchungen getrockneter luftgefullter Gewebe mit dem Ultropak Beitr path Anat 92, 270.

Fischer, E (1935), Lymphgefäßuntersuchungen an serosen Häuten mit Luftfullungsmethoden (Das Verhalten der Netzlymphgefaße bei chronischen Entzündungen ) Verh. deutsch pathol Ges 28, 223

Fischer, E & H Kaiserling (1956), Die experimentelle lymphogene allergischhyperergische Appendicitis. Virchows Arch. 297, 146

[illegible]

Evans, B. & A. Ochsner (1954), The gross anatomy of the lymphatics of the human pancreas, Surgery 36, 177.

Evans, C. L. (1945), Principles of human physiology. 9th ed. Churchill, London

Evans, H. (1907/08), The blood-supply of lymphatic vessels in man. Am. J. Anat. 7, 195

Everett, N. B., W. E. Garrett & B. S. Simmons (1954), Lymphatics in iron absorption and transport. Am. J. Physiol. 178, 45.

Everhardt, J. K. & A. M. Jacobs (1938), Chylothorax. Review of literature and report of a case in newborn infant. J. Pediatrics 15, 558.

Exner, A. (1903), Über die durch intraperitoneale Adrenalin-Injection verursachte Verzögerung der Resorption von in den Magen eingeführten Giften. Arch. exp. Path. 50, 313

Exton-Smith, A. N. & D. J. Crochett (1957), Nature of oedema in paralyzed limbs of hemiplegic patients. Brit. Med. J. 1280

Fahr, T. (1925), Pathologische Anatomie des Morbus Brightii in Hdb. d. spez. Path. Anat. u. Hist. Henke-Lubarsch, Bd. 6/T. I, 156. Springer, Berlin.

Fahr, T. (1942), Über Glomerulosklerose. Virchows Arch. 309, 16.

Falloise, A. (1902), Le travail des glandes et la formation de la lymphe. Bull. Acad. Roy. Sc. de Belg. 4, 945.

Farber, S. (1937a), Studies on pulmonary edema; consequences of bilateral cervical vagotomy in rabbit. J. Exp. Med. 66, 397.

Farber, S. (1937b), Studies on pulmonary edema. II. The pathogenesis of neuropathic pulmonary edema. J. Exp. Med. 66, 405.

Farkas, G. (1927), Studien über den kolloid-osmotischen Druck des Serums. Z. exp. Med. 53, 666.

Farkas, G. (1938), Der Kolloiddruck des Blutes in Bennhold—Kylin—Rusznyák: Die Eiweißkörper des Blutplasmas. Dresden u. Leipzig.

Fauteux, M. (1946), Treatment of coronary disease with angina by pericoronary neurectomy combined with ligation of great cardiac vein, case report. Amer. Heart J. 31, 260

Favaro, G. (1905/06), Ricerche intorno alla morfol. ed allo sviluppo dei vasi, [illegible] Veneto di Sc. Lett. [illegible] io alla circolazione [illegible] ulare riguardo agli Urodeli. Atti dell'Ac. Sc. venet.-trentino-istriana Cl. I 3, 122.

Favaro, G. (1908), Ueber den Ursprung des Lymphgefäßsystems. Anat. Anzeiger 33, 75.

Favilli, G. (1939), Effetti di alcune sostanza capillaro-attive sulla diffusione del fattore R nei tessuti, imbizione della diffusione per azione della adrenalina e dell'ormone ipofisario vasocostrittore (vasopressina). Riv. di pat. sper. 23, 113.

Fazio, A. Connessioni linfatiche tra fegato e cistifellea e loro importanza nella patogenesi della sindrome epatocolecistica. Ann. Ital. Chirurg. 29, 345, Fasc. VI.

(Fedorova, V. N.) Федорова, В. Н. (1953) Определение двуокиси кремния в лимфатических узлах методом микросожидания (озоления) при силикозе. Арх. патологи 15, 41

Fehr, A. (1931), Zur Kenntnis der Verodung des Ductus thoracicus. Virchows Arch. 279, 265

Fejfar, Z. & J. Brod (1951), Význam neurohumorálních činitelů pro vznik obĕchových zmen při srdeční nedostatečnosti. Sborník lékarski 53, 99.

Feldberg, W., E. Schilf & H. Zernik (1928), Das Verhalten des Pfortaderdruckes nach Injektion von Histamin und Pepton in den Kreislauf des Hundes. Pflugers Arch. 220, 738.

[illegible] W. & E. Schilf (1930) [illegible] Bedeutung [illegible] fatica dei [illegible]

Foldi, M. & E. Kallee (1957), A pajzsmirigy nyirokérrendszerének autoradiographiás vizsgálata (Autoradiographic examination of the lymphatic system of the thyroid), Orv. Hetil (Hungarian), 37.

Foldı, M, J Kepes, M. Papp, I. Rusznyák & Gy. Szabó (1954), A tudő

bution to the data concerning the pathogenesis of pulmonary oedema II. Provo-

Acta Med. Hung (In the press.)

Foldı, M., A Korányı & Gy Szabó (1947), Az anaemiás oedemák keletkezésének mechanizmusa (Mechanism of the genesis of anaemic oedemas), Orvosok Lapja (Hungarian), 6, 545.

Földı, M, A. Korányı & Gy Szabó (1948), Über die Entstehung anämischer Ödeme Acta Med Scand 129, 486

Foldı, M, M Papp, F. Soltı, & E. Koltay (1957), A nátriumretentio kérdése generalizált lymphoedemában (The problem of sodium retention in generalized lymphoedema) MTA V Oszt kozlem (Hungarian), 8, 287

Foldı, M. & Gy. Romhányı (1953 a), A vese nyırokkeringésének jelentősége hydronephrosisban (Significance of renal lymph circulation in hydronephrosis) Orv Hetil. (Hungarian), 94, 315

Foldı, M & Gy. Romhányi (1953 b), Untersuchungen uber den Lymphstrom der Niere Acta Med Hung 4, 323

& Gy Szabó (1954a), A szív nyır ardiac lymph circulation). MTA

& Gy. Szabó (1954b), Über die I Med Hung 6, 61.

Gy Szabó & A Temesvári

(1955), Wirkung von venoser und Lymphstauung auf die Herzmuskulatur. Acta Med Hung 7, 33

(1950), A nyırokrendszer system in inflammatory

), A vese nyırokérrend- (Lymphatic apparatus lomerulosclerosis). MTA

Foldı, M, I. Rusznyák, & Gy. Szabó (1948), Passiv tubularis cukorrediffusio (Passive tubular glucose rediffusion) Orv Hetil (Hungarian), 89, 369

... nalis ... Arb. physiol Anst. Leipzig 4, 24.

Flemming, W. (1885), Studien über Regeneration der Gewebe. Arch mikr. Anat. 24, 50.

Florey, H. (1926), Capillary permeability. J. Physiol. Proc. 61, 1.

Florey, H. (1926/27), Observations on the contractility of lacteals. Part. I. J. Physiol. 62, 267.

... refere ... F ... tum. J. ... and ...

Florey, H. W & H. M. Carleton (1926/27), Rouget cells and their function. Proc. Roy. Soc. s. B. 100, 23.

Florey, H & H M. Carleton (1927), Mammalian lacteal, its histological structure in relation to its physiological properties. Proc. Roy Soc. ser B 102, 110.

Florey, H. & L. J. Witts (1928), Absorption of blood from the peritoneal cavity, Lancet I. 1323.

Foglia, V. G. (1939), Papel vital de los corazonas linfaticos del sapo. Rev. Soc. Argent. de Biol. 15, 97

Foglia, V. G., E. Braun-Menendez (1939), El registro gráfico de la actividad de los corazones linfáticos Rev. Soc. Argentina de Biol. 15, 178.

Foglia, V. G, E Braun-Menendez (1939), Influencias térmicas, endocrinas y farmacológicas sobre los corazones linfáticos. Rev. Soc. Argentina Biol 15, 235

Foglia, V G., R. Gerschman (1939), Trastornos humorales y circulatorios por destrucción de los corazones linfaticos del sapo. Rev. Soc. Argentina de Biol 15, 113.

Foglia, V. G., R. L Zwemer (1943), Trastornos de distribución de fluidos por destrucción de corazones linfáticos. Rev. Soc. Argentina de Biol. 19, 341.

Fohmann, V. (1832), Mémoires sur les vaisseaux lymphatiques avec les veines et sur les vaisseaux absorbants du placenta et du cordon ombilical J. Desoèr, Liége.

Fohmann, V. (1833), Mémoires sur les vaisseaux lymphatiques de la peau, ... Liége.

... into ... ngés ... pest, Dissertation in Hungarian.

Földi, M. (1954a), Die Entstehung des kardialen Ödems. Zeitschr ärztl. Fortbildung. 48, 337.

Földi, M. (1954b), Beitrag zur Frage der Entstehung des Lungenodems. Munch Med. Wochenschr. 96, 1548.

Földi, M. (1954c), A Dupuytren-kór új kezelése hyaluronidase-novocain infiltratióval (A new treatment of Dupuytren's disease with hyaluronidase-novocain infiltration). Orv Hetilap (Hungarian), 95, 182.

Földi, M. (1955), Unsere neuen Ergebnisse in der Physiologie und Pathologie der Lymphströmung. Zeitschr ges. inn Med. 10, 483.

Földi, M., H Jellinek, & Gy. Szabó (1955 a), Vizsgálatok a pajzsmirigy nyirokérrendszeréről (Investigations into the lymphatic system of the thyroid), MTA V. oszt. közl. (Hungarian), 6, 13.

... das Lymph- ... tok a nyirok- ... othel sejtjei- ... of protein ... in the endothel ... Oszt. Közl. (Hungarian), 5, 89.

Földi, M., H. Jellinek, I Rusznyák & Gy. Szabó (1955), Eiweißspeicherung in den Endothelzellen der Lymphkapillaren. Acta Med. Hung 7, 211.

Follett, A. E. (1918), Preparation and some properties of hyaluronic acid from umbilical cord of the pig. J Biol. Chem. 176, 177.

Fogelman, M. J., P. O. Montgomery & C. A. Moyer (1952), Internal water exchange rates following hemorrhage in splenectomized dogs. Am. J. Physiol. 169, 94

Forbes, G B, R W. Deisher, A. M. Perley & A F Hartman (1950), Effect of hyaluronidase on the subcutaneous absorption of electrolytes in humans Science 111, 117.

Forfota, E & S. Karády (1937), Über die biologische Allgemeinwirkung der

Fowler, N. O., R. N. Westcott & R. C. Scott (1951), Pulmonary artery diastolic pressure; its relationship to pulmonary arteriolar resistance and pulmonary "capillary" pressure. J. Clin Investig 31, 72

Fox, J. P. (1936), The localization and concentration of blood borne antibodies and colloidal dyes in areas of inflammation of various ages J Immunol. 31, 293.

Franke, K (1912), Über die Lymphgefäße der Lunge, zugleich ein Beitrag zur Erklärung der Baucherscheinungen bei Pneumonie Deutsche Zeitschr. Chirurg. 119, 107.

Fränkel, A. (1892), Über primären Endothelkrebs (Lymphangitis proliferativa) der Pleura Verhandl 11 Kongr Inn Med Wiesbaden

Frankenthal, L. (1931), Über Ascites chylosus Arch klin Chir. 164, 248

(Frautchi, V. H.) *Фраучи, В X* (1938), Об анатомическом обосновании лимфогематогенных метастазов Вопр клинической и экспериментальной онкологии 104

(Frautchi, V. H.) *Фраучи, В X* (1948), Лимфо-венозные анастомозы у человека Хирургия 11, 12.

Frédéricq, H (1936), Action des substances autonomomimétriques sur les coeurs lymphatiques de la grenouille C R Soc Biol 121, 160

Freeman, W. L (1942), Lymphatic pathways from the intestine in the dog. Anat Record 82, 543

Fresen, O (1942), Lipoideiweißkristalle im interstitiellen Gewebe der Niere. Virchows Arch 308, 344

Fresen, O (1943), Die Bedeutung des Lymphgefäßsystems der menschlichen Niere Klin Wschr 22, 664

Frey, H. (1863), Untersuchungen uber die Lymphgefäße des Darmkanals. W Engelmann, Leipzig

Frey (1873), Das Mikroskop

Frey, W (1947), Die exzessive tubuläre Ruckresorption Schweiz med Wochenschr 77, 75

Frey, W. (1951), Handbuch d inneren Medizin 4 Aufl Bd 8. Nieren u. ableitende Harnwege Springer, Berlin—Gottingen—Heidelberg.

Friedman, M, S O Byers, C. Omoto (1956), Some characteristics of hepatic lymph in the intact rat Am J. Physiol 184, 11

in th

cefal

Friou, G. J & H A. Wenner (1947), On the occurrence in the human serum of an inhibitory substance to hyaluronidase produced by a strain of hemolytic streptococcus J Infect Dis. 80, 185

Fuchs, S. & H. Popper (1938), Blut und Saftstromung in der Niere Erg. inn. Med Kinderheilk 54, 1.

Fuhrman, F. A & H. H. Ussing (1951), A characteristic response of the isolated frog skin potential to neurohypophyseal principles and its relation to the transport of sodium and water J Cell & Comp. Physiol 38, 109

Foldi, M, I. Rusznyák & Gy Szabó (1949a), Vizsgálatok a beteg vesehám működéséről (Investigations regarding the function of diseased renal epithelium) Magyar Belorv Arch (Hungarian), 2, 20.

Foldı, M, I. Rusznyák & Gy Szabó (1949b), A nyirokkeringés szerepe a phlebohypertoniás oedemák keletkezésében (Role of lymph circulation in the origin of phlebohypertonic oedemas) Magyar Belorv. Arch. (Hungarian), 2, 332.

Foldı, M, I. Rusznyák & Gy Szabó (1949c), Van-e az antistinnek antıhyaluronidase hatása? (On the antıhyaluronidase effect of antistine). Orv Hetil. (Hungarian), 90, 493.

Foldı, M, I. Rusznyák & Gy. Szabó (1949d), A hyaluronidase hatása a víz és oldott anyagok felszívódására (Effect of hyaluronidase on the absorption of water and dissolved substances). Orv. Hetil. (Hungarian), 90, 707

Foldi, M, I. Rusznyák & Gy. Szabó (1949e), Passive tubuläre Glukoserediffusion. Acta Med Scand 134, 225.

Foldı, M., I Rusznyák & Gy Szabó (1950), On the inhibitory effect of antistin on hyaluronidase Biochim. Biophys. Acta. 4, 579.

... a nyirokáramlásra (Effe ... arian), 4. 159.

... keringés szerepe a hypa ... circulation in the formation of hypalbuminaemic oedemas) Magy. Belorv. Arch (Hungarian), 4, 161.

Foldı, M., I. Rusznyák & Gy. Szabó (1951c), A pangásos oedemák keletkezésének mechanizmusa (Mechanism of the origin of congestion oedemas) Magy Belorv. Arch. (Hungarian), 4, 166

Foldı, M, I. Rusznyák & Gy. Szabó (1952), The role of lymph-circulation in the pathogenesis of edema. Acta Med Hung. 3, 259

... adék tároló ... lymphatic ... ngés idegi szabályozásának kérdése. Dibenamin hatása a nyirokáramlásra (Neural regulation of lymph circulation. Effect of dibenamine on lymph flow) Orv. Hetil (Hungarian), 91, 739

... ro- ... ok- ... az ... fu- sion of fluids and macromolecules in the interstitial space) MTA V. Oszt. Kozl (Hungarian), 4, 519

Foldı, M, I Rusznyák, Gy. Szabó & Zs Magyar (1954), Untersuchungen uber die Funktion der Lymphkapillaren Ausbreitung der Flüssigkeit und der Makromoleküle im Interstitium Acta Med Hung 6, 229.

Foldı, M, I Rusznyák, Gy. Szabó & E Vágó (1951), A plasma antıhyaluronidase titere vesebajos és cardiális oedemás betegeken (Anti-hyaluronidase titre of the plasma in patients with renal disease and cardiac oedema) Magy. Belorv.

... Magyar (1953), ... ık útja az inter- ... ph capillaries. I. ... lymph vessels).

... Gynec & Obst. 101, 25.

Gergely R., Z. Zsebök & M. Foldi (1956), Die diagnostischen Anwendungsmöglichkeiten der Lymphangiographie. Fortschr. Röntgenstr. 85, 175.

Gerhardt, D. (1897), Zur Pathogenese des Ikterus. Verh Congr. inn. Med. 15, 160

Gerhartz, H. (1925), Chemie der Lymphe in Hdb. der Biochemie des Menschen und der Tiere, hrsg v. C Oppenheimer, 2. Aufl. 4. Bd.

Gerota, D. (1896a), Ueber eine Verbesserung des Quecksilberinjektionsapparates für Lymphgefäße. Anat. Anz. 12, 35

Gerota, D. (1896b), Zur Technik der Lymphgefäßinjektion. Eine neue Injektionsmasse der Lymphgefäße. Polychrome Injektion. Anat. Anz. 12, 216 und Verh d. Anat Ges 1896, p. 151, 152.

Gersh, I. & H. R. Catchpole (1949), The organisation of ground substance and basement membrane and its significance in tissue injury, disease and growth Am. J. Anat. 85, 457.

Gerster, R. (1876), Ueber die Lymphgefäße des Hodens Ztschr Anat. Entwicklungsg 2, 36

Gibson, T. & J. S. Tough (1955), A simplified one-stage operation for the correction of lymphedema of the leg Arch. Surgery 71, 809

Gilman, T. & A. C. Ivy (1947), A histological study of the participation of the intestinal epithelium, the reticulo-endothelial system and the lymphatics in iron absorption and transport Gastroenterology 9, 162.

Ginsberg, S. (1899), Ueber die Abfuhrwege des Zuckers aus dem Dünndarm. Pflügers Arch. 44, 306.

Girgensohn, H. (1952), Die Bedeutung der Lymphgefäße in der Nierenpathologie. Ztschr. Kreislaufforsch. 41, 111.

Glass, A (1928), Über Beeinflussung des Adrenalin-Lungenodems durch experimentelle Verletzungen des Hirnstammes und des Sympathikus. Arch. exp. Path. 136, 80.

Glasunow, M. (1936), Durchspulungsversuche mit Trypanblau an uberlebenden Aorten. Virchows Arch. 261, 837.

Glenn, W W [illegible]

Glenn, W W. L., H H. Gilbert & C. K. Drinker (1943), The treatment of burns by the closed-plaster method, with certain physiological considerations implicit in the success of this technique. J Clin Invest 22, 609

Glenn, W. W L, D. K. Peterson & C. K Drinker (1942), The flow of lymph from bu[illegible] lymph [illegible]

Gl[illegible] ronidase [illegible]

Glick, D. & M. L Grais (1948), Concerning the alleged occurrence of hyaluronidase in skin. Arch Biochemistry 18, 511

Glick, D, R A. Good, V C. Kelley, R J. Winzler & J. W. Mehl (1949), Lack o[illegible] mucoproteins in blood serum. I[illegible]

[illegible] zyme systems. XIII. Effect of c[illegible] sition by human serum Proc. S[illegible]

[illegible] phologische und systematische Untersuchungen uber die serose Entzundung der Leber, nebst Beitragen experimenteller Leberschädigungen durch Bakterien, Bakterientoxine und mechanisch-operative Eingriffe. Virchows Arch. 312, 64.

Glynn, L E, H P. Himsworth & O. Linder (1948), Experimental production and development of diffuse hepatic fibrosis ("portal cirrhosis"). Brit. J. Exp. Path. 29, 1.

Golden, A. & I. F. Tullis (1949), Diffuse interstitial fibrosis of the lungs Min. Surgeon. 105, 130

Fulleborn, F (1895), Beiträge zur Entwicklung der Allantois der Vögel Med Inaug. Diss. Berlin, P. 17.
Funaoka, S. (1930), Der Mechanismus der Lymphbewegung. Arb. aus der III. Abt. des Anat. Inst d. Kaiserl. Univ. Kyoto, Sér. D. 1, 1.
Funaoka, S., R. Tachikawa, C. Yamaguchi & S. Fujita (1930), Kurze Mitteilung uber die Rontgenographie des Lymphgefäßsystems sowie über den Mechanismus der Lymphstromung. Arb. III. Abt Anat. Inst. Kaiserl. Univ. Kyoto Sér. D 1, 11.
Further, H. (1913), Beiträge zur Kenntnis der Vogellymphknoten Jena Zeitschr. Naturwiss. 43, 359.
Furuta, W. (1948), The histologic structure of the lymph node capsule of the hilum. Anat. Rec 102, 213.
Gable, E. (1926), Über die Wirkung der sympathischen Innervation auf die Zirkulation und den Stoffaustausch in den Muskeln. Z exper. Med. 51, 728.
Gabrio, B. W. & K. Salamon (1950), Distribution of total feritin in intestine and mesenteric lymph nodes of horses after iron feeding. Proc Soc. Exp. Biol Med 75, 124
Galkin, W. S (1930), Über die Bedeutung der Nasenbahnen fur den Abfluß aus dem Subarachnoidalraum. Zeitschr ges. exp Med 72, 65
Galkin, W. S. (1930), Zur Methodik der Injektion des Lymphsystems vom Subarachnoidalraum aus (Bedeutung der Injektionsstelle.) Zeitschr. ges. exp. Med. 74, 595
Gamble, J. E & H D. Patton (1951), Pulmonary edema and haemorrhage induced by hypothalamic lesions in rats Science 113, 626
Gamble, J. E & H. D Patton (1953), Pulmonary edema and haemorrhage from preoptic lesions in rats Am J. Physiol. 172, 623
Gamble, J. L. & S. G. Ross (1924/25), The factors in the dehydration following pyloric obstruction. J. Clin Invest. 1, 403.
Gamble, J L. (1952), Chemistry, anatomy, physiology and pathology of extracellular fluid. Harward Univ. Press. Cambridge Mass.
Gamna, C. (1927), Sur la clinique et l'étiologie de la splénogranulomatose

Garrey, W E. & W. R. Bryan (1935), Variations in the white blood cells counts. Physiol. Rev. 15, 597.
Gärtner, G. & F. Romer (1891), Über die Einwirkung von Bakterienextrakten auf den Lymphstrom. Wiener med. Blätter. 14, 654.
Gastaldi, A (1947/48), Osservazioni sulla linfocrinia della tiroide Mon. Zool Ital. 56, Suppl. 58.
Gaupp (1896), in A. Eckers-Erwiedersheim: Anatomie des Frosches auf Grund einiger Untersuchungen durchaus neu bearbeitet 3 Aufl. Braunschweig
Gellért, A , M. Poberai, I Nagy, S Nagy & J. Lippai (1957), Összehasonlító szövettani vizsgálatok a nyirokerek falszerkezetére vonatkozóan. I. A ductus thoracicus falának szoveti felépítése (Comparative histological investigations regarding the structure of the wall of lymph vessels. I. Histological structure of the

branen fur Farb-
eaction to adren-
ion of adrenalin.
.peratur auf die
die Sehnen und
Fascien der Skelettmuskeln. Arb. Physiol. Anst. Leipzig 5, 53
Gergely R. & Z. Zsebők (1956), De la lymphangiographie Presse Médicale. 64, 2200.

Gergely, R., Z. Zsebök & M. Földi (1956), Die diagnostischen Anwendungsmöglichkeiten der Lymphangiographie. Fortschr. Röntgenstr. 85, 175.

Gerhardt, D. (1897), Zur Pathogenese des Ikterus. Verh. Congr. inn. Med. 15, 460.

Gerhartz, H. (1925), Chemie der Lymphe, in: Hdb. der Biochemie des Menschen und der Tiere hrsg. v. C. Oppenheimer, 2. Aufl. 4. Bd.

Gerota, D. (1896a), Ueber eine Verbesserung des Quecksilberinjektionsapparates für Lymphgefäße. Anat. Anz. 12, 35.

Gerota, D. (1896b), Zur Technik der Lymphgefäßinjektion. Eine neue Injektionsmasse der Lymphgefäße. Polychrome Injektion. Anat. Anz. 12, 216. und Verh. d. Anat. Ges. 1896, p. 151, 152

Gersh, J. & H. R. Catchpole (1949), The organisation of ground substance and basement membrane and its significance in tissue injury, disease and growth. Am. J. Anat. 85, 457.

Gerster, R. (1876), Ueber die Lymphgefäße des Hodens. Ztschr. Anat. Entwicklungsg. 2, 36

Gibson, T. & J. S. Tough (1955), A simplified one-stage operation for the correction of lymphedema of the leg. Arch. Surgery 71, 809.

Gilman, T. & A. C. Ivy (1947), A histological study of the participation of the intestinal epithelium, the reticulo-endothelial system and the lymphatics in iron absorption and transport. Gastroenterology 9, 162.

Ginsberg, S. (1899), Ueber die Abfuhrwege des Zuckers aus dem Dünndarm. Pflügers Arch. 44, 306.

Girgensohn, H. (1952), Die Bedeutung der Lymphgefäße in der Nierenpathologie. Ztschr. Kreislaufforsch. 41, 111.

Glass, A. (1928), Über Beeinflussung des Adrenalin-Lungenödems durch experimentelle Verletzungen des Hirnstammes und des Sympathikus. Arch. exp. Path. 136, 80.

Glasunow, M. (1936), Durchspülungsversuche mit Trypanblau an überlebenden Aorten. Virchows Arch. 261, 837

Glenn, W. W., S. L. Cressor, F. X. Bauer, F. Goldstein, O. Hoffman & J. E. [illegible] burns by the closed-plaster method, with certain physiological considerations implicit in the success of this technique. J. Clin. Invest. 22, 609

Glenn, W. W. L., D. K. Peterson & C. K. Drinker (1942), The flow of lymph from [illegible] lym[illegible] roni[illegible]

Glick, D. & M. L. Grais (1948), Concerning the alleged occurrence of hyaluronidase in skin. Arch. Biochemistry 18, 511.

Glick, D., R. A. Good, V. C. Kelley, R. J. Winzler & J. W. Mehl (1949), Lack of identity of hyaluronidase inhibitor and certain mucoproteins in blood serum. Proc. Soc. Exp. Biol. Med. 71, 412.

Glick, D. & M. Kaufman (1950), Mucolytic enzyme systems XIII. Effect of certain compounds on hyaluronidase and its inhibition by human serum. Proc. Soc. Exp. Biol. Med. 74, 279

[illegible] Experimentell-morphologische und systematische [illegible] tzundung der Leber, nebst Beiträgen experi- [illegible] Bakterien, Bakterientoxine und mechanisch- [illegible] 312, 64

Glynn, L. E., H. P. Himsworth & O. Linder (1948), Experimental production and development of diffuse hepatic fibrosis ("portal cirrhosis"). Brit. J. Exp. Path. 29, 1.

Golden, A. & I. F. Tullis (1949), Diffuse interstitial fibrosis of the lungs. Min. Surgeon. 105, 130.

Goldmann, E. (1913), Vitalfärbung am Zentralnervensystem. Beitr. z. Physiologie des Plexus chorideus. Berlin.

(Goldstein, D. E.) *Гольдштейн Д. Е.* (1950), Материалы к изучению лимфатической системы методом прижизненной лимфографии. Казань 1949 Сов реф обозр. 3/35.

Gollan, F. (1956), Lymphatic drainage of large particles. Federation Proc. 15, 79.

Goltz, F. (1863), Neue Tatsachen uber den Einfluß der Nerven auf die Herzbewegung. Centralbl. med. Wissensch. 119, 40.

Gomori, P. (1941), Collapsus és shock (Collapse and shock). Orvosképzés — továbbképző előadások (Hungarian), 54.

Gomori, P. (1953), A keringés elégtelensége (Circulatory insufficiency), E u *Kiadó, Budapest (Hungarian).*

Gomori, P., S. Kisfaludy & L. Urai (1948), A capillarisok áteresztőképessége hemiplegiás betegeken (Capillary permeability in hemiplegic patients). Orv. Hetil. (Hungarian), 89, 389.

Gonzales-Oddone, M. V. (1946), Bilirubin, bromsulfalein, bile acids, alkaline phosphatase and cholesterol of thoracic duct lymph in experimental regurgitation *jaundice. Proc. Soc. Exp. Biol. Med. 63, 144.*

Goodwin, W. E., J. J. Kaufman (1956a), The renal lymphatics, I. Review of some of the pertinent literature. Urological Survey, 6, 305.

Goodwin, W. W., J. J. Kaufman (1956b), The renal lymphatics and hydronephrosis. Surgical Forum. 6, 632.

Goodwin W. E., J. J. Kaufman (1956c), Renal lymphatics, II. Preliminary experiments *J. Urol. 76, 702.*

Gorinstein, H. L. (1913), Weitere experimentelle Studien uber Herzbeutelresorption. Beitr. z. klin. Chir. 86, 229.

Gorlin, R., B. Lewis, F. W. Haynes, R. J. Spiegl & L. Dexter (1951), Factors regulating pulmonary "capillary" pressure in mitral stenosis IV. Am. Heart J. 41, 834

*lorid okozta* tudő-
onary oedema due
49
progression osmo-
de l'hypertension

rotéinurie. Journ.
d'Urologie. 59, 693

Graham, B. F. & R. A. Cleghorn (1951), Changes in the circulating leukocytes following electrically induced convulsions in man. J. Clin. Endocrinol. 11, 1469.

Graham, E. A. (1921), Influence of respiratory movements on formation of pleural exudates. J. A. M. A. 76, 784.

Grais, M. L. & D. Glick (1948), Mucolytic enzime systems; Inhibition of ... J. Invest. Dermat. 11, 259.

Lymph-
schemic
.94.
ascites.
J. Missouri M. A. 20, 214

Gregersen, M. I. & R. A. Rawson (1942/43), The disappearence of T-1824 and structurally related dyes from the blood stream. Am. J. Physiology 138, 698.

Grindlay, J. H., J. C. Cain, J. L. Bollman & E. V. Flock (1948), Experimental studies on liver and thoracic duct lymph. Minnesota Medicine 31, 654

(Gryaznova, A. V.) *Грязнова, А. В.* (1955), К вопросу о лимфо-венозных *анастомозах между грудным протоком и непарной веной у собаки* Архив анат. гистол. эмбриол. 32.

Gross, H. (1914), Die Lymphstauung und ihre Produkte. Deutsche Ztschr. Chir. 127, 1.

Gross, H. (1916), Mechanismus der Lymphstauung. Deutsche. Ztschr. Chir. 138, 318.

Gross, L., G. Silverman & A. M. Master (1936), Electrocardiographic changes following coronary sinus occlusion in dogs heart. Amer. Heart. J. 11, 734.

Groot, S. B. (1912), Kritische und experimentelle Untersuchungen über das Entstehen und Verschwinden von Lymphdrüsen. Deutsche Ztschr. Chir. 119, 428.

Grotte, G. (1955), Passage of dextran molecules of varying sizes from blood to lymph. Scand J. Clin. Lab. Invest. 7, 59.

Grotte, G., R. C. Knutson & J. L. Bollman (1951), The diffusion of dextrans of different molecular sizes to lymph and urine. J. Lab. & Clin. Med. 38, 577.

Guerra, F. (1946), Hyaluronidase inhibition by sodium salicylate in rheumatic fever. Science 103, 686.

Guttmann, L. (1936), Physiologie und Pathologie der Liquormechanik und Liquordynamik in Bumke, O. & O Foerster: Hdbuch der Neurologie. Bd. VII/2. Springer, Berlin.

Haas, E. (1946a), On the mechanism of invasion. I. Antinvasin I., an enzyme in plasma. J. Biol. Chem. 163, 63.

Haas, E. (1946b), On the mechanism of invasion II Proinvasin I an enzyme in pathogenic bacteria and venoms. J Biol Chem. 163, 89

Hadfield, G. (1939), The primary histological lesion of regional ileitis. Lancet 2, 773.

Hadidian, Z. & N. W. Pirie (1948), Preparation and some properties of hyaluronic acid from human umbilical cord. Biochem. J 42, 260

Hagentorn, A. (1901), Fall elephantiatischer Verdickung des Unterschenkels mit diffuse- [illegible] 51, 795.

Hahn [illegible] se I. Demonstration o [illegible] nd triphenylmethane c [illegible]

Hahn, P F. & E L Carothers (1951), Radio-active metallic gold colloids coated with silver and their distribution in the lung and its lymphatics following intrapulmonary administration; therapeutic implications in primary lung and bronchiogenic tumors. Brit J Cancer 5, 400

Hahn, P. F., L L. Miller, F. Robscheit-Robbins, W. F. Bale & G. H Whipple (1944), Peritoneal absorption, red cells labelled by radio-iron hemoglobin move promptly from peritoneal cavity into circulation. J Exp. Med 80, 77

Hahn, P. F , G. Rouser, H. Brommitt, J. Moorehead & E. L. Carothers (1952), The drainage of radio-active silver colloids by the lymphatics following intrapulmonary administration in dogs. J Lab & Clin Med. 39, 624.

Haitinger (1938), Fluoreszenz-Mikroskopie. Leipzig.

Hakanson, E Y. & D. Glick (1948), Mucolytic enzyme systems III. Inhibition of hyalu [illegible]

Hal [illegible] ittingensi factae [illegible]

Hal [illegible]

Halmágyi, D. (1954), Personal communication

Halmágyi, D. & F. Robicsek (1954), Personal communication

Hamburger, F (1945), Epinephrine and tissue permeability. Yale J. Biol Med. 17, 479

Hamburger, H J. (1893), Untersuchungen uber die Lymphbildung, insbesondere bei Muskelarbeit. Z Biol. 12, 143

Hamburger, H. J (1895), Über die Regelung der osmotischen Spannkraft von Flussigkeiten im Bauch und Perikardialhöhle Ein Beitrag zur Kenntnis der Resorption Arch Anat u Physiol. Physiol. Abt. 281.

Hamburger, H. J. (1902), Osmotischer Druck und Ionenlehre. Bergman, Wiesbaden

Hamman, L. & A. R. Rich (1944), Acute diffuse interstitial fibrosis of the lungs. Bull Johns Hopkins Hosp 74, 177

Hammarsten, O. (1926), Lehrbuch der physiologischen Chemie. 11. Aufl. Bergman, Munchen

[illegible]

tion) titers in sera of patients with rheumatic fever, streptococcal infection and others. Am. J. Med. Sci. 217, 174.

Harris, E. & H. M. Marvin (1927), The innervation of mammalian capillaries by vasoconstrictor sympathetic nerves. Heart 14, 135.

Harris, J. A, C. M. Jackson, D. G Paterson & R. E. Scammon (1930), The measurement of man. The University of Minnesota Press Minneapolis.

Harris, R., A. J. Martin & H. S. Williams (1952), Correlation of skin temperature and circulatory changes in muscle and subcutaneous tissue of the hand during trunk heating. Clinical Science 11, 429.

Harris, T. N., S. Harris, A M. Dannenberg Jr. & J. L Hollander (1950), Streptococcal antihyaluronidase titers in the sera of patients with rheumatoid arthritis and glomerulonephritis Ann. Int. Med. 32, 917.

Hartroft, W. S. (1952), Liver Injury. Transact. 11th Conf. Josiah Macy Jr. Found. New York.

Harvey, W. H. (1906/07), Experimental lymphocytosis. J. Physiol 35, 115.

Harwey, W. (1628), Exercitatio nova de motu cordis et sanguinis Frankfurt.

Hashimoto, H. (1925/26), Blood chemistry in acute histamine intoxication J. Pharmacol. & exper. Therap. 25, 381.

Hass, H. (1936), Die Architektur der Lymphgefäße der Lebercapsel in ihren Beziehungen zur Bindegewebsstructur und Flussigkeitsstromung. Virchows Arch 297, 384.

[illegible] phgefäß-system [illegible] v 2, 159

[illegible] ic lymph and [illegible]

[illegible] gefäße. Verh. Deut [illegible]

[illegible] of hyaluronidase as influenced by prolonged local treatment with adrenal cortical extract. Proc. Soc. Exp. Biol. Med 75, 361.

Hayes, M A. & R M. Bridgeman (1951), Dermal spreading of hyaluronidase as influenced by prolonged local treatment with certain steroid hormones. Proc. Soc. Exp. Biol. Med. 77, 597.

[illegible] ntent [illegible] ology. 101, 223

Haynes, F W. (1932 b), Further observations on the rapidity of passage of [illegible]

Physiol. 97, 52.

Hechler, F. H. (1897), Über den diagnostischen Wert der Lymphdrüsenschwellung in den Oberschlusselbeingruben, besonders in der linken, bei Magenkrebs. Berlin Diss

Hechter, O. (1948), Lymphocyte discharge from isolated rabbit spleen by adrenal cortical extract. Endocrinology 42, 285.

Hechter, O (1950), Mechanism of spreading factor action. Ann. New York Acad Sci. 52, 1028.

Hechter, O. S, K. Dopkeen & M H. Yudell (1947), The clinical use of hyaluronidase in hypodermoclysis J. Pediat. 30, 645.

Hechter, O. & S. Johnson (1949), In vitro effect of adrenal cortical extract [illegible]

Hedinger, E. (1907), Über die Kombination von Morbus Addisoni mit Status lymphaticus. Frankf. Z. Path. 1, 527

Heidenhain, R. (1888), Beiträge zur Histologie und Physiologie der Dünndarmschleimhaut. Pflügers Arch. 43, 1.

Heidenhain, R. (1891), Versuche und Fragen zur Lehre von der Lymphbildung. Pflügers Arch. 49, 209

Heidenhain, R (1896), Bemerkungen und Versuche betreffs der Resorption in der Bauchhöhle. Pflügers Arch. 62, 320

Heim, J. W. (1933), On the chemical composition of lymph from subcutaneous vessels. Am. J. Physiol. 103, 553.

Hellems, H. K., F. W. Haynes, L. Dexter & T. D Kinney (1948), Pulmonary capillary pressure in animals estimated by venous and arterial catheterization Am. J. Physiol 155, 98.

Heller, A. (1896), Ueber selbständige rhythmische Contractionen der Lymphgefäße bei Säugethieren. Centralbl. med. Wissenschaft. 7, 545.

Hennequin, L. (1935), Les battements des coeurs lymphatiques de la grenouille. C. R. Soc. Biol. 119, 40.

Hennequin, L., L Merklen, H. Renaudin (1935), A propos du synchronisme des coeurs lymphatiques homolatéraux chez la grenouille. C R Soc Biol. 119, 781

Henry, C. G. (1933), Studies on the lymphatic vessels and on the movement of lymph in the ear of the rabbit. Anat. Record. 57, 263

Henry, J. P., I. Klain, E Movitt & J. Meehan (1946), On the effects of acute anoxia on the permeability of the capillaries in the human arm Feder Proc. 5, 44

Henry, J P., J. Goodman & J. Meehan (1947), Capillary permeability in relation to acute anoxia and to venous oxygen saturation J. Clin Invest. 26, 1119

Hensen, V. (1868), Ueber die Nerven im Schwanze der Froschlarven Arch mikrosk. Anat 4, 111.

Heppner, G. T (1934), Bilateral chylothorax and chyloperitoneum J A M A. 102, 1294.

Hering, E (1867), Über den Bau der Wirbeltierleber Arch mikr. Anat. 3, 88.

Herring, P. T & S Simpson (1906), The relation of the liver cells to the blood vessels and lymphatics Proc Physiol Soc. Lond 23.

Herrmann, R (1951), Troubles circulatoires pulmonaires aigus par traumatismes craniens Ann méd lég. 31, 411

Hertzler, A E. (1901), The morphogenesis of the stigmata and stomata occurring in peritoneal and vascular endothelium Trans. Am Microscop. Soc. 23, 63.

Hertzler, F (1915), The development of fibrous tissues in peritoneal adhesions. Anat Record 9, 83

Hewson, W [illegible]

[illegible]

Hint, H. C & G Thorsen (1947), A micro method for determination of dextran in blood Acta chem Scand 1, 808

Hirschler, Á & K. Buday (1899 a), A chylosus hasvízkór egy esetéről (A case of chylous ascites) Orv. Hetil (Hungarian), 33, 424, 438, 451.

Hirschler, A. & K Buday (1899 b, c), További jelentés a chylosus ascitesről (A new contribution to chylous ascites). Orv Hetil. (Hungarian), 33, 107, 451.

His, W. (1863), Ueber das Epithel der Lymphgefäßwurzeln und uber die v. [illegible] Zool. 13, 455.

[illegible] ystems. Zeitschr Anat. u [illegible]

[illegible] den Ikterus. Ein Beitrag [illegible] 541.

[illegible] ffee (1941), The relation- [illegible] Med. 73, 109

[illegible] und der Gewebe. 6. Aufl. W. Engelmann, Leipzig.

Hochstetter (1903), Entwicklung des Lymphgefäßsystems. In· O. Hertwigs Handb. d. vgl. u exp. Entwicklungslehre d. Wirbeltiere. Bd. 3. G Fischer, Jena.

Hoffmann, F. (1740), Opera omnia physico-medica. Genevae.

Hollander, W., P Reilly, B. A. Burrows (1956), Lymphatic flow in human subjects as indicated by the disappearance of $I^{131}$ labelled albumin from the subcutaneous tissues. J. Clin. Invest. (Proc.) 35, 713.

Holler, G. (1943), Gelbsuchtsformen. Die epidemischen Gelbsuchtskrankheiten *Urban & Schwarzenberg, Berlin.*

Holman, C, B. McSwain & J. N. Beal (1941), Swelling of the upper extremity following radical mastectomy Surgery 15, 757.

Holmes, J H. & E E. Painter (1947), Role of extracellular fluid in traumatic shock in dogs Am. J Physiol. 148, 201.

Holmgren, H. & O. Wilander (1937), Beitrag zur Kenntnis der Chemie und Funktion der Ehrlichschen Mastzellen Z Mikr.-anat. Forsch. 42, 242.

Holzmann, K. (1955), Klinische Elektrocardiographie. 3. Aufl. G Thieme, Stuttgart.

Homans, J. (1940), Lymphoedema of limbs. Arch. Surgery 40, 232

Hooker, D. R. (1920), The functional activity of the capillaries and venules. Am J. Physiol. 54, 30.

Hopf, G. (1937), *Die Quaddelresorptionszeit als Ausdruck verschiedenartiger physiologischer Vorgänge am Hautorgan. Dermat. Wschr 104, 513.*

Hoppe-Seyler, F. (1881), Physiologische Chemie. A. Hirschwald, Berlin.

Hopps, H. C. & J H Lewis (1947), Studies on capillary permeability as affected by anoxemia. Am J Path. 23, 829.

Horne, J. van (1652), *Novus ductus chyliferus.* Nunc primum delineatus, descriptus eruditorium examini expositas. Lugd. Batav.

Horst, H G. (1952), Zur Frage des neuralen Lungenodems beim Epileptiker. Ärztl Wschr 7, 35

Horstmann, E. (1952), Über die funktionelle Struktur des mesenterialen *Lymphgefäße* Morphol. Jahrb. 91, 483.

Horton, B. T. (1927), Lymphatics of stomach with special reference to pyloric block Proc. Staff. Meet. Mayo Clin. 2, 312

Hotovy, R. (1939), Beiträge zur Physiologie und Toxikologie der Lymph*herzen bei Amphibien. Pflugers Arch. 242, 180*

Howell, W H. (1914), The coagulation of lymph. Am J. Physiol. 35, 483.

[illegible] Recherches sur les vaisseaux lymphatiques des sélaciens. [illegible] Forellen- [illegible] nol. Jahrbuch [illegible]

Hoyer, H. & S Udziela (1912), Untersuchungen über das Lymphgefäßsystem von Salamander larven. Morphol. Jahrb. 44, 535.

*Hudack, S. S. & P. D. McMaster (1932), The gradient* of permeability of the skin vessels as influenced by heat, cold and light. J. Exp. Med. 55, 431.

Hudack, S. S. & P. D McMaster (1933), The lymphatic participation in human [illegible] phenomena. J. Exp. Med 57, 751.

Hueck, W. (1920), Über das Mesenchym. Die Bedeutung seiner Entwicklung und seines Baues für die Pathologie. Beitr. Pathol. Anat. 6, 330.

Huf, E. (1935), Versuche über den Zusammenhang zwischen Stoffwechsel, Potentialbildung und Funktion der Froschhaut. Pflügers Arch 235, 655

Hughes, W. T. & A. J. Carlson (1908). The relative hemolytic power of serum and lymph under varying conditions of lymph formation. Am. J Physiol 21, 236.

Hukuda, K. & G. Takanayagi (1933), On the lymphagogue action of lymphoglandular extract. Nagoya Journ. med. Soc. 7, 75.

Humphrey, J. H. & R Jaques (1953), Hyaluronidase; correlation between biological assay and other methods of assay. Biochem. J. 53, 59

Hungerford, G. F. & W. O. Reinhardt (1948), The immediate effect of adrenalectomy on the lymphocyte content of rat thoracic duct lymph. Anat. Record 100, 746.

Hungerford, G. F., O. W. Reinhardt & C. H. Li (1952 a), Effects of cortisone and hydrocortisone on the number of thoracic duct lymphocytes. Blood, 7, 1125

Hungerford, G. F., W. O Reinhardt & C H Li (1952), Effects of pituitary and adrenal hormones on the number of thoracic duct lymphocytes Blood 7, 193.

Hung, R. (1917) Vasodilator reactions Am. J. Physiol 43, 197

Hunter, W. (1762), Medical commentaries. London.

Hunter, W. (1784), Two introductory lectures to his last course of anatomical lectures at his theatre in Windmill, London

Huntington, S. (1911), The anatomy and development of the systemic lymphatic vessels in the domestic cat. Mem. Wistar Inst Anat Biol Philadelphia

Huntington, G. S. (1914), The development of the mammalian jugular lymph sac, of the tributary primitive ulnar lymphatic and of the thoracic duct from the [illegible]
a [illegible]
in [illegible]
[illegible]
main lymph channels of the cat in their relation to the venous system Anat. Record 1, 36.

Huntington, G. S. & C F. W. McClure (1908), The anatomy and development of the jugular lymph sacs in the domestic cat. Am. J. Anat 2, 1.

Huntington, G. S (1908), The genetic interpretation of the development of the mammalian lymphatic system. Anat Record. 2, 19

Hülse, W. (1918), Untersuchungen uber Inanitionsodeme. Ein Beitrag zur Pathologie des Ödems. Virchows Arch. 225, 234.

Hürthle, K. (1894), Beiträge zur Kenntnis des Secretionsvorganges in der Schilddrüse. Pflügers Arch 56, 1

Huzella, T. (1929), Der Entwicklungsmechanismus und die organisatorische Bedeutung des Gitterfasersystems Arch. Entwickl Mechan. 116, 430

Hyatt, R. E., G. H. Lawrence & J R. Smith (1955), Observations on the origin of ascites from experimental hepatic congestion. J. Lab. & Clin Med 45, 274.

Hyatt, R. E. & J. R Smith (1954), The mechanism of ascites A physiologic appraisal. Am. J. Med. 16, 434.

Hyman, C. & R Chambers (1943), Effect of adrenal cortical compounds on edema formation of frog's hind limbs Endocrinology. 32, 310.

Hyrtl, J. (1860), Der Ursprung der Chylusgefäße. Öster Ztschr Prakt. Heilk. 6, 337.

Inatsku, N. (1928), Studies on the inflammatory exudation. II Studies of inflammatory exudation with reference to its relation to sympathetic nerves. Transact. Jap Path Soc. 18, 341.

Irminger and Frey (1866), Ein Beitrag zur Kenntnis der Gallenwege in der Leber des Säugethiers. Zeitschr Wiss. Zool. 16.

Isayama, S (1924/25), Über die Geschwindigkeit des Flussigkeitsaustausches zwischen Blut und Gewebe. Ztschr. Biol. 82, 101.

Issayeff (1894), Untersuchungen uber die kunstliche Immunität gegen Cholera Ztschr Hyg u. Infektionskrankh. 16, 287.

Isayama, S. (1924), Über die Stromung der Lymphe bei den Amphibien Zeitschr. f. Biol. 82, 91.

Ito, T. (1926), Über den Flüssigkeitsaustausch zwischen Lymphe und Blut beim Frosch. Pflugers Arch. 213, 718.

Iversen, P. & F. Nakazawa (1927), Über die Biochemie des Filtrationsödems. Biochem. Z. 191, 307.

Iwanow, G. (1927), Über die Abflußwege aus den submeningealen Räumen des Ruckenmarks Zeitschr. ges. Exp. Med. 58, 1.

Iwanow, G. (1929), Über die Abflußwege aus den Subarachnoidalräumen des Gehirns und Ruckenmarks und die Methodik ihrer intravitalen Untersuchung Zeitschr. ges exp. Med. 64, 356.

(Ivanov G F.) *Иванов, Г. Ф.* (1936), О некоторых формах циркуляции лимфы вне сосудов Арх. биол наук 48, 214

(Ivanov G F.) *Иванов, Г. Ф.* (1939) Об интраадвентициальных системах бронхов Арх биол. наук 53, 123.

(Ivanov G F) *Иванов, Г Ф.* (1953) К критике некоторых теорий путей лимфооттока Арх анат. гист и эмбриол. 30/5, 78.

Iwanow, G. & K. Romodanowsky (1927), Über den anatomischen Zusammenhang der cerebralen und spinalen submeningealen Raume mit dem Lymphsystem. (I. Mitteilung) Methodik u. wichtigste Beobachtungen. Zeitschr. ges. Exp Med 58, 596

Jackson A. S. (1937) ...

... Virchows Arch ..., 331.

Jäger, ... die Beteiligung der L... 19, 552

Jagic, ... Gallenkapillaren. Ein ... hose. Zieglers Beitr. 33, 302.

Jancsó, N. (1914), A histaminhatások láthatóvá tétele a szovetekben (Demonstration of histamine effects in the tissues) Magy Belorv. Arch. (Hungarian), 42, 367.

Jancsó, N (1955), Speicherung. Verlag d Ung. Akademie d. Wiss, Budapest.

Jancsó, N. & A Jancsó-Gábor (1952a), Zellulare Verteilung und Speicherungsmechanismus des »Bayer 205« (Germanin) in den Geweben. Acta Physiol Hung. 3, 537

Jancsó, N. & A. Jancsó-Gábor (1952b), Sichtbarmachung von Immunreaktionen in den Geweben. Acta Physiol. Hung. 3, 555.

Jankowski, K. W. (1883), Über die Bedeutung der Gefaßnerven fur die Entstehung des Ödems. Virchows Arch. 93, 259

Japelli, G. & G. D'Errico (1907/08), Beiträge zur Lymphogenese V. Über die physiko-chemischen Eigenschaften der postmortalen Lymphe Z. Biol 1, 1.

Jarisch, A. & H. Richter (1939), Zentrogenes Lungenodem. Klin. Wschr. 18, 1440.

... ges. ... their ...

... lymphatiques chez les oiseaux. C. R. Soc. Biol 46, 499.

Jolly, J (1919), Sur les organes lymphoïdes des céphaliques des batraciens. C. R. Soc. Biol. 82, 200.

Jolly, J. (1919), Sur l'existence chez les batraciens d'organes lymphoïdes pouvant être considérés comme des ébouches des ganglions lymphatiques C R. Soc Biol. 82, 201.

Jores, A. (1906), Über experimentelles, neurotisches Lungenodem. Deutsch Arch klin. Med. 87, 389.

(Jossifow, G. M.) Иосифов, Г М (1904), Начало грудного протока и его расширение. Труды обш. научн. мед и хир при Харков ун-те 1

Jorpes, J E. (1936), Heparin in the treatment of thrombosis London

Joseph, D. R. (1914/15), On the convulsant action of acid fuchsin in cardiectomized frogs after removal of the anterior lymph hearts J. Pharmacol and Exper Therap. 6, 83.

Jossifow, G. M (1903), Zur Lehre vom Lymphgefäßsystem der Kaulquappe, des Frosches und der Eidechse. Mém. Acad Sc St. Pétersbourg 15, 20.

(Jossifow, G. M.) Иосифов, Г. М. (1904), Начало грудного протока и его расширение. Труды обс. научн мед. и хир при Харков ун-те 1

Jossifow, G. M. (1905a), Sur les voies principales et les organes de propulsion de la lymphe chez certains poissons osseux. C R. Soc. Biol 58, 205

Jossifow, G. M. (1905b), Hat die Placenta Lymphgefäße? Arch Anat u Physiol. Anat. Abt. 333.

Jossifow, G. (1906), Der Anfang des Ductus thoracicus und dessen Erweiterung Arch Anat. u. Physiol. Anat. Abt. 68.

(Jossifow, G. M ) Иосифов, Г М (1914), Лимфатическая система человека Томск.

Jossifow, G. M. (1930), Das Lymphgefäßsystem des Menschen Jena

Juhász, J., J. Baló & G Vendrey (1953), Kísérleti glomerulosclerosis (Experimental glomerulosclerosis) Kísérl Orvostud. (Hungarian), 5, 196

Julesz, M. & al. (1954), Personal communication.

Kaindl, F., E Mannheimer, P Polsterer & B Thurnher (1957), Darstellbarkeit und funktionelles Verhalten der Lymphgefäße in menschlichen Extremitaten Zeitschr f Kreislaufforschung. 46, 115

Kaindl, F, E. Mannheimer, P. Polsterer & B Thurnher (1957), Zur Ätiologie von Ödemen in Extremitäten Zeitschr f. Kreislaufforschung 46, 296

Kaiserling, H. (1938), Untersuchungen zur Frage der Beziehungen des Nervensystems zur allergisch-hyperergischen Entzündung. Virchows Arch. 301, 111

Kaiserling, H. (1940), Lymphgefäße und Lymphangitis der Niere Virchows Arch 306, 322

Kaiserling, H. (1942), Die Ausbreitungsformen der Nierenlymphbahninfekte und die lymphogene Nephrose. Virchows Arch 309, 561.

Kaiserling, H & T. Soostmeyer (1939), Die Bedeutung des Nierenlymph- [illegible]

[illegible]

...ampmeier, O F. (1912), The value of the injection method in the study of lymphatic development Anat. Rec 6, 223.

Kampmeier, O. F. (1931), Ursprung und Entwicklungsgeschichte des Ductus thoracicus nebst Saccus lymphaticus jugularis und Cisterna chyli beim Menschen Morpholog. Jahrb. 67, 157

Kaplan, A , M. Friedman & H E. Krieger (1943), Observations concerning the origin of renal lymph. Am. J. Physiol. 138, 553.

Karády, J. & M. Foldi (1943), Adatok a „physiologiás albuminuria" keletkezésének mechanizmus[illegible] logical albuminuria

Karczag, L. & [illegible] durch den Sympat[illegible]

Karsner, H T. & C E Swanbeck (1920/21), The removal of particulate matter from the plasma J Med Research 42, 91.

Ztsc'

Zeitschr f Biol. 82, 91.

Ito, T (1926), Über den Flüssigkeitsaustausch zwischen Lymphe und Blut beim Frosch. Pflugers Arch. 213, 718.

Iversen, P & F Nakazawa (1927), Über die Biochemie des Filtrationsodems Biochem Z. 191, 307

Iwanow, G (1927), Über die Abflußwege aus den submeningealen Räumen des Ruckenmarks. Zeitschr ges. Exp Med. 58, 1

Iwanow, G. (1929), Über die Abflußwege aus den Subarachnoidalräumen des Gehirns und Ruckenmarks und die Methodik ihrer intravitalen Untersuchung. Zeitschr ges exp Med. 64, 356

(Ivanov G F.) *Иванов, Г Ф. (1936), О некоторых формах циркуляции* лимфы вне сосудов. Арх. биол. наук 48, 214

(Ivanov G F) *Иванов, Г Ф.* (1939) Об интраадвентициальных системах бронхов Арх. биол наук 53, 123

(Ivanov G. F.) *Иванов, Г Ф. (1953) К критике некоторых теорий путей* лимфооттока Арх. анат гист и эмбриол 30/5, 78.

Iwanow, G. & K. Romodanowsky (1927), Über den anatomischen Zusammenhang der cerebralen und spinalen submeningealen Räume mit dem Lymphsystem. (I. Mitteilung) Methodik u wichtigste Beobachtungen. Zeitschr. ges Exp. Med. 58, 596.

Jackson, A S (1937), Regional enteritis Surg, Gynec & Obst. 65, 1.

Jacobi, W. (1921), Pharmakologische Wirkungen am peripherischen Gefäß- ... Veranderung der Perme- ... xp Path 88, 333

... l Ein Beitrag zur Ent- ... im Pfortadersystem. Virchows Arch 299, 551.

*Jager, E. (1937b), Milzbau und Kreislaufstorung. II Teil. Über die Beteiligung der* ... Milzbau Virchows Arch. 299, 552

Jagic, ... gische Histologie der Gallenkapillaren Ein ... und der biliären Cirrhose. Zieglers Beitr 33, 302.

Jancsó, N. (1914), A histaminhatások láthatóvá tétele a szovetekben (Demonstration of histamine effects in the tissues). Magy. Belorv Arch. (Hungarian), 42, 367.

Jancsó, N (1955), Speicherung. Verlag d Ung Akademie d Wiss, Budapest.

Jancsó, N. & A Jancsó-Gábor (1952a), Zellulare Verteilung und Speicherungsmechanismus des »Bayer 205« (Germanin) in den Geweben. Acta Physiol Hung. 3, 537.

Jancsó, N. & A Jancsó-Gábor (1952b), Sichtbarmachung von Immunreaktionen in den ...

Jankowski, K ... Gefaßnerven fur die Entstehung des Ödem ...

Japelli, G & ... mphogenese V. Über die physiko-chemischen Eigenschaften der postmortalen Lymphe Z. Biol. 1, 1.

Jarisch, A. & H Richter (1939), Zentrogenes Lungenodem Klin Wschr. 18, 1440

Jasienski, G (1935), Les lymphatiques du rein sain J. d'Urol. 40, 97.

Jafimow, K. (1928), Zur Frage der Genese des epileptischen Anfalls. Zeitschr. ges. Exp Med. 62, 662.

Job, T. T. (1918), Lymphatico-venous communications in the common rat and their significance. Am. J Anat. 24, 467.

Jolly, J. (1909), Sur une disposition spéciale de la structure des ganglions lymphatiques chez les oiseaux C. R. Soc. Biol 46, 499.

Jolly, J. (1919), Sur les organes lymphoïdes des céphaliques des batraciens. C. R. Soc. Biol. 82, 200.

Kiss, F. & T. Orbán (1950), Újabb adatok a szem vérkeringéséhez (New data concerning the eye's blood circulation) Szemészet (Hungarian), 87, 71.

Kisselew, E S (1951), Dissertation, Leningrad.

Kisselew, J. (1869), Über die Lymphgefäße der Leber. Zbl Med. Wiss. 7, 117.

Kitmanow, K. A. (101), Über die Nervenendigungen in den Lymphgefäßen. Anat. Anz 19, 369.

Klein, E (1875), The anatomy of the lymphatic system. London.

Klemensiewitz, R. (1912), Die Pathologie der Lymphstromung In· Krehl—Marchands Hdb. d. allg. Path. Anatomie Abt. I, Bd. II. Hirzel, Leipzig

Kling, C. A. (1904), Studien über die Entwickelung der Lymphdrüsen beim Menschen. Arch mikr Anat. 63, 575.

Kneeland, Y. & H F Smetana (1940), Current bronchopneumonia of unusual character and undetermined etiology. Bull Johns Hopkins. 67, 229.

Knisely, M H. (1936), Spleen studies II Microscopic observations of the circulatory system of living traumatized spleens, and of dying spleens. Anat. Rec. 65, 131.

Knisely, M. H., E. H. Bloch & L Warner (1948), Selective phyagocytosis I. Microscopic observations concerning the regulation of the blood flow through the liver and other organs and the mechanism and rate of phagocytic removal of particles from the blood K. Danske Videnskabernes Selskab, Biologiske Strifter, København 4, 7.

[illegible] terior lymph

[illegible] к рефлексов

[illegible] орецепторов сосудов кишечника почек и почечных лоханок на лимфоток Изв АН Казахской ССР, сер физиол 73/2, 111.

(Kokhanina, M Y) *Коханина, М Я* (1949b), Рефлекторно-гуморальная регуляция лимфотока. Изв АН Казахской ССР, сер физиол 73/2, 11

(Kokhanina, M. Y) *Коханина, М Я.* (1951), Рефлексы с селезенки на лимфоток. Сов мед реф. обозр 8/43

Kodama, M. (1925), Beiträge zur Pathogenese des Ikterus Zieglers Beiträge. 73, 187.

Koenig, K H. & R. R Koenig (1949), Studies of the pathogenesis of ammonium pulmonary edema Am J Physiol 158, 1

Kollert, V & W Starlinger (1924), Albuminurie und Bluteiweißbild Z. klin. Med 99, 426.

Kolliker, A (1854), Handbuch der Gewebslehre des Menschen Mikr. Anat. Bd. II/2 a

Kolliker, A. (1863), Handbuch der Gewebelehre 4 Aufl Leipzig.

Koniges, H G & M Ottó (1937), Studies on the filtration mechanism of the intestinal lymph and on the action of acethylcholine on it and on the circulation of the intestinal villi Quart J Exp Physiol 26, 319

Kolossow, A (1893), Ueber die Struktur des Peritoneal- und Gefäßepithels (Endothels) Arch mikr Anat 42, 318.

Kondoleon, E (1912), Die Lymphableitung, als Heilmittel bei chronischen Oedemen nach Quetschung München, Med. Wschr. 59, 525

Ko [illegible] klinische Untersuchungen uber den osmotis [illegible] Ztschr Klin. Med 33, 1.

Ko [illegible] funktionelle Pathologie und Therapie der N [illegible]

Katsuki, T (1921), Ein Beitrag zur Frage der *metastatischen Carcinombildung* der Milz. Fukaoka-Iwadaigakuzasshi. 17, 56.

Katzenstein, R., E. Mylon & M C. Winternitz (1943), Toxicity of thoracic duct fluid after release of tourniquets applied to hind legs of dogs for production of shock. Am. J. Physiol. 129, 307.

Kaufmann, F. (1911), Lehrbuch der speziellen pathologischen Anatomie. 6. Aufl. Reiner, Berlin.

(Kazanski, A. A.) *Казанский, А А* (1951), Роль лимфатической системи в патогенезе острого гнойного плеврита. Хирургия 12/37

Kelemen, E., J Iványi & M. Majoros (1951), Hyaluronidase limb-edema test on rat. Acta Med. Hung. 2, 201.

Kelemen, E. & al. (1953), Personal communications

Kerpel-Fronius, Ö. (1936), *Salzmangelzustände und chloroprieve Azotämie.* Erg. inn. Med u Kinderheilk. 51, 623

*Key & Retzius* (1875/76), *Studien in der Anatomie des Nervensystems* und Bindegewebes Nordsted et Sohner, Stockholm

Kim, K S, J L. Bollman & J H Grindlay (1956), Metabolism of endogeneous lipid of the intestine Am. J. Physiol 184, 445

Kimmelstiel, P. & C Wilson (1936), Intercapillary lesions in the glomeruli of the kidney. Am J Path. 12, 83.

*Kimura, K & F. Nakazawa* (1930), *Studien uber den kolloid-osmotischen* Druck des Bluts im normalen und pathologischen Zustand IV. Kolloid-osmotischer Druck des Bluts bei experimenteller Nierenschädigung Tohoku J Exp Med. 16, 352.

Kindwall, J. A (1927). A supra-vital study of the cells in the lymph stream of the rabbit. Bull. Johns Hopkins Hosp 40, 39.

Med 68, 63

Schilddruse.

Sept. 1893)

idt 24, 260

Antidiabeti-

of outlining

lymphatic truncs at operation Clinical Sci 11, 13.

Kinmonth, J. B., G W Taylor & R. K. Harper (1955), Lymphangiography A technique for its clinical use in the lower limb Brit. Med Journ I 940

Kisfaludy, S. & P Braun (1954), Quantitative Bestimmung der Aminosäuren durch Papierchromatographie Ztschr. ges inn Med. 9, 699

Kishi, S (1932), Die Lymphstromung ableitenden Wege nach der Unterbrechung des Ductus thoracicus in der Brusthohle. Arb. Anat Inst Kaiserl. Univ. Kyoto Sér. D I, 50

Kiss, F. (1930), Topographic relationship between the nerve plexuses and lymph nodes of the abdomen Arch. Surg 21, 405

...atiques et

...ion of the

collagenous fibres). Ideggyógyászati Szemle (Hungarian), 7, 73

Kubik, I., T. Vizkelety & J. Bálint, Pulmonary segments in regional lymph nodes. Anatomical Record. (In the press)
Kubo, H (1932), Beitrag zur Kenntnis der Lymphknotenfunktion. (Auf Grund von Untersuchungen an paraaortalen- und Gekroselymphknoten.) Virchows Arch. 283, 593.
Küchmeister, H. (1949), Die Wirkung des Rutin auf die Capillarpermeabilität. Klin Wschr. 27, 297.
Küchmeister, H. (1953), Die Klinik der Capillarfunktionen. Ergebnisse d. inn. Med. u Kinderheilk. Neue Folge 4, 163.
Küchmeister, H. (1954), Hesperidinphosphat als Hyaluronidaseinhibitor. Klin Wschr. 32, 299
(Kudrin, I. S.) *Кудрин, И. С* (1953), К анатомии лимфатической системы матки. Труды Ленинград сан.-гиг мед ин-та 17, 186
Kühn, H. A. & G. Hildebrand (1951), Untersuchungen über die Leberlymphe. Klin. Wschr. 29, 785.
Kühn, H. A (1952), Über den Übertritt von Gallenbestandteilen in die Leberlymphe. Klin. Wschr 30, 662.
Kühn, H. A. (1944/1947), Die formale Pathogenese der Hepatitis epidemica nach Untersuchungen an Leberpunctaten Beitr pathol. Anat. 109, 589.
Kulenkampff, H. (1950), Acini und Lymphsinus in der Schilddruse des Neugeborenen Zeitschr Anat u. Entwicklungsgeschichte 115, 82
Kumagai, L. F. & T. F. Dougherty (1951), Stress-induced lymphocytosis in adrenalectomized mice. Federation Proc. 10, № 1.
Kumita (1909), Über die Lymphgefäße der Nieren und Nebennierencapseln. Arch. Anat. Phys. Abt. Anat. 49
Kumita (1909), Über die Lymphbahnen des Nierenparenchyms Arch Anat u. Physiol. Anat. Abt. 99
Kunkel, A. (1875), Untersuchungen über den Stoffwechsel und die Leber Würzburg
Kunkel, A (1886), Die Leber als Ausscheidungsorgan fremdartiger Blutbestandtheile Stahel, Würzburg
Kunos, I & A Temesváry (1954), A szív vénás keringési zavaranak hatása a szívizomra (Effect of disturbed cardiac venous circulation on the heart musculature) Magyar Belorv Arch. (Hungarian). 7, 10
Kusmine, K (1905), Untersuchung uber die Entstehung und die Eigenschaften der Lymphe, 6 Mitteilung Ueber den Einfluß der Lymphagoga (Lebergifte) auf die Leber Zeitschr Biol 46, 554
Kusnetzowsky, N (1925), Über den Einfluß lokaler Reize auf den Prozeß der Vitalfärbung Ztschr. ges exp Med 44, 646
Kyber, E. (1870) *Ueber die Milz des Menschen und einiger Säugethiere* Arch. mikr. Anat. 6, 541
Kylin, E (1921), Eine Modifikation meines Kapillardruckmessers sowie Referat der Secherschen Nachuntersuchungen mit diesem Messer Zentralbl inn. Med. 42, 785.
Kylin, E & H Pein (1931), Studien uber den kolloidosmotischen (onkotischen) Druck. X Vereinfachte Onkometrie fur den klinischen Gebrauch Z. ges exp Med 76, 549
Kytmanof (1901), Ueber die Nervenendigungen in den Lymphgefäßen der Saugetiere Anat. Anzeiger 19, 369
Laas, E (1932), Die hyalinen Tropfen in der Niere Virchows Arch 286, 426.
Laborit, H & P Morand (1946), Sur un test de résorption utilisable dans le diagnostic du choc et dans son évolution Mém Acad Chir. 72, 65.
Lake, B. J., W. I Simmonds & A W. Steinbeck (1953), The effect of foreign plasma on capillary permeability in unsensitized animals Austral. J. Exp. Biol 31, 55
Lambert, P. P. & T. Grégoire (1952), Perméabilite glomérulaire et protéinurie. J d'Urol 58, 775
Lambert, P P, T Grégoire C. de Heinzelin & C de Braucourt (1952), Hémodynamique glomérulaire et excrétion de l'hémoglobine. Arch intern Physiol 60, 506

Kósa G., J. Foldes & Á. Orosz (1957), Lymphatic connections between the nasal mucosa and intracranial space. Acta Morph Acad. Sci Hung 7, 361.

(Kositsyn, I I.) *Косицын, И И* (1946), О развитии лимфатической системы щитовидной железы у человека Об трудов Ижевск гос мед ин-та 5, 181

(Kositsyn, I. I.) *Косицын, И. И* (1946), О возможности впадения лимфатических сосудов в вены без перерыва их в лимфатических узлах. Об. трудов Ижевск гос мед ин-та 5, 175.

(Kositsyn, I. I.) *Косицын, И И.* (1953), Нервный аппарат лимфатических узлов Вопр морфологии 2, 190

Kovách, A. (1952), Personal communication.

Kovách, A , J. Menyhárt, Kiss, S., A. Erdélyi & E. Kovách (1954), Dibenamin hatása ischaemiás shockban (Effect of dibenamine in ischaemic shock). Lecture held in Hungarian.

Kowalewsky, A. (1895), Études sur le système lymphatique des insectes et myriapodes Bull. d'Acad Sci de St. Pétersbourg Sér 5, 2, 1.

(Kovanov, K. V ) *Кованов, К В.* (1954), Лимфатическая система как регулятор кровяного давления Труды Всес общ. физиол. биохим и фармак. Москва

Krause, A K. (1925), Human resistence to tuberculosis at various ages of life Amer. Rev Tbc 11, 303.

Krause, W (1855), Zur Physiologie der Lymphe. Ztschr. rationelle Med. 7, 148

Krieger, H., O W. Holden, C. A Hubay, M. W. Scott, J. P. Storoaash & H. L. Friedell (1950), Appearance of protein tagged with radio-active iodine in thoracic duct lymph. Proc Soc. Exp Biol Med. 73, 124

Krogh, A (1918/19), The supply of oxygen to the tissues and the regulation of the capillary circulation. J Physiol. 52, 457

Krogh, A (1922), Anatomy and physiology of capillaries. Yale Univ Press. New Haven

Krogh, A (1929), Anatomie und Physiologie der Capillaren II. Aufl. Springer, Berlin

[illegible] absorp- [illegible] oedema [illegible]

Krogh, A. & P B Rehberg (1922), Sur l'influence de l'hypophyse sur la tonicitié des capillaires. C R. Soc. Biol 87, 461

Krogh, A. & F. Nakazawa (1927), Beiträge zur Messung des kolloid-osmoti- [illegible] luid [illegible] loid- [illegible] óth, [illegible] aft- kreislautes der Nasenschleimhaut mit dem der Schädelhohle bzw. dem Liquor Acta Physiol. Hung 6, 56.

Krompecher, Ö (1910), Zur Anatomie, Histologie und Pathogenese der gastrischen und gastrointestinalen Sklerostenose. Zieglers Beitr. 49, 384.

Kryloff, E. (1927), Zur Frage der eitrigen Entzundung des Ductus thoracicus Virchows Arch. 266, 1.

Kubik, J (1950), Adatok a nyirokkeringés mechanizmusához (Data regarding the mechanism of lymph circulation) Kísérl Orv. tud. (Hungarian), 2, 182.

Kubik, I. (1952), Die hydrodynamischen und mechanischen Faktoren in der [illegible] Acta Morph Hung 2, 95 [illegible] Mesenterium [illegible] lymph circu- [illegible]

Lauson, H. D., S. E. Bradley & A. Cournard (1944), The renal circulation in shock. J. Clin Investig 23, 381.

Lauson, H. D., C. W. Forman, H. McNamara, G. Matter & H. L. Barnett (1954), The effect of corticotropin (ACTH) on glomerular permeability to albumin in children with the nephrotic syndrome. J. Clin Investig. 33, 657

Lawrentjew, A. P. (1927a), Über die Nerven der Lymphgefäße in der Bauchhöhle. Anat. Anz. 63, 268.

Lawrentjew, A. P. (1925/26), Zur Lehre von der Innervation des Lymphsystems. Ueber die Nerven des Ductus thoracicus beim Hunde. Anat. Anz. 60, 475.

Lawrentjew, A. P. (1927b), Zur Lehre von der Innervation des Lymphsystems; über die Nerven der Lymphgefäße in der Bauchhöhle. Anat. Anz. 62, 430.

Lazarus-Barlow, W. S. (1895/96), Contribution to the study of lymph-formation with special reference to the parts played by osmosis and filtration. J. Physiol. 19, 418.

Leathes, J. B. (1895/96), Some experiments on the exchange of fluid between the blood and tissues. J. Physiol. 19, 1.

Lee, F. C. (1922), Establishment of collateral circulation following ligation of the thoracic duct. Bull. John Hopkins Hosp 33, 21.

Lee, F. C. (1923/24), Some observations on lymph pressure. Am J Physiol. 67, 498.

Lee, F. C. (1925), On the lymphvessels of the liver. Contrib. Embryol. 74, 65.

Leenhardt, P. & R. Colin (1957), L'exploration lymphatique «in vivo». Presse méd. 65, 1534.

Lehnartz, (1910), Einführung in die chemische Physiologie. 4. Aufl. Springer, Berlin.

Leigh, O C. (1935), Lymph formation during glandular activity Am J. Physiol. 112, 657.

Leiter, L. (1931), Experimental nephrotic edema. Arch. Int Med. 48, 1.

Lemon, W. S & G M. Higgins (1932), Absorption from pleural cavity of dogs; lymphatic system. Am. J. Sci 184, 846.

Lepeschkin, E. (1951), Modern Electrocardiography. Williams and Wilkins, Baltimore.

Leriche, R. & J Kunlin (1934), Traitement immédiat des phlébites postopératoires par l'infiltration novocaïnique du sympathique lombaire. Presse Méd 42, 1481.

Levitan, B. A. (1940), Action of vitamin P on the stability of connective tissue ground structure Am J Physiol 137, 422

Lewandowsky, M. H. (1900), Zur Lehre von der Cerebrospinalflussigkeit. Ztschr. Klin. Med. 40, 480.

Lewandowsky, M H (1907), Die Funktionen des zentralen Nervensystems Fischer, Jena.

Lewachew, S. W (1886a), Recherches sur la production de la lymphe. Arch. slaves biol Par. 2, 56.

Lewachew, S. W. (1886b), Études comparatives sur l'influence des deux ordres des nerfs vasomoteurs sur la circulation de la lymphe, sur leur mode d'action et sur le mécanisme de la production lymphatique C. R Acad. de Sc. 103, 75.

Lewis, B M & R Gorlin (1952), Effects of hypoxia on pulmonary circulation of the dog Am J Physiol 170, 574.

Lewis, F. T (1906), The development of the lymphatic system in rabbits. Am. J. Anat. 5, 95.

[illegible]

Heart 11, 119.

Lewis, T. & R. Grant (1925), Observations upon reactive hyperaemia in man. Heart 12, 73.

Lambert, R. K. & H. Gremels (1926), On the factors concerned in the production of pulmonary edema. J. Physiol. 61, 98.

Landerer, Q. (1884), Die Gewebsspannung in ihrem Einfluß auf die örtliche Blut- und Lymphbewegung. Vogel, Leipzig.

Landis, E. M. (1925/26), The capillary pressure in frog mesentery as deter- [illegible]

[illegible] in human skin. Heart 15, 209

[illegible] an mesentery [illegible]

[illegible] cle and skin [illegible]

Landis, E. M (1934), Capillary pressure and capillary permeability Physiol Rev. 14, 404.

Landis, E. M (1946), Capillary permeability and the factors affecting the composition of capillary filtrate. Ann. N. Y. Acad Sc. 46, 713.

Landis, E. M & J. H. Gibbon Jr. (1933), The effects of temperature and of tissue pressure on the movement of fluid through the human capillary wall. J. Clin. Investig 12, 105.

Landis, E. M., L. Jonas, M. Angevine & W. Erb (1932), The passage of fluid and protein through the human capillary wall during venous congestion J. Clin. Investig. 11, 717.

Lange, L. (1877), Ueber den Magenkrebs und seine Metastasen. Med. Diss. Berlin. 27.

Langendorff, (1899), Beiträge zur Kenntnis der Schilddrüse. Arch Physiol Supp. Band. 218

Langendorff, O (1906), Neue Untersuchungen uber die Tätigkeit des Lymphherzens. Pflugers Arch 115, 533.

Langhans, Th. (1892), Über Veränderungen in den peripherischen Nerven bei Kachexia thyreopriva des Menschen und Affen sowie bei Kretenismus Virchows Arch. 128, 368.

Langohr, J. L., L. Rosenfeld, C. R. Owen & O. Cope (1949), Effect of therapeutic cold on circulation of blood and lymph in thermal burns; experimental study. Arch. Surg. 59, 1031.

Lanz (1911), Eroffnung neuer Abfuhrwege bei Stauung in Bauch und in den unteren Extremitäten. Zentralbl. Chir. 38, 153.

[illegible] -ma- tique [illegible] nd uber [illegible] -ri- mentelle und theoretische Grundlagen zur Therapie der Phosgenerkrankung... Z. exper. Med. 13, 200.

Lasker, S. E., Ch L. Fox Jr & J. M. Winfield (1954), Redistribution of total body albumin and electrolytes in mice after thermal burns Federation *Proc.* 13, 84.

Latta, H. (1947), Pulmonary edema and pleural effusion produced by acute alfa-naphthyl-thiourea poisoning in rats and dogs. Bull. John Hopkins Hosp. 80, 181.

Laufer, S. (1935), Unterbindung der Coronarvenen, pathologisch-anatomische Untersuchungen. Verh. deutsch Ges. Kreislaufforsch. 223.

Luisada, A. A. (1940), The pathogenesis of paroxysmal pulmonary edema. Medicine 19, 475.

Luisada, A. A. (1943), The treatment of paroxysmal pulmonary edema, with special reference to formes occurring under wartime conditions. Exp. Med & Surg. 1, 22.

Luisada, A. A. & S. J. Sarnoff (1944), Studies of paroxysmal pulmonary edema. A new experimental method. Proc. Soc. Exp. Biol Med 57, 279

Luisada, A. A. & S. J. Sarnoff (1946), Paroxysmal pulmonary edema consequent to stimulation of cardiovascular receptors. II. Effect of intra-arterial and intravenous infusions Am. Heart J. 31, 270.

Lurie, M B (1959), Mechanisms affecting spread in tuberculosis. Ann. New York. Acad. Sc. 52, 1074.

Macallum, A. B. (1891/92), On the demonstration of the presence of iron chromatin by microchemical methods. Proc. Roy Soc. 50, 277

Macallum, A. B (1894), On the absorption of iron in the animal body. J. Physiol. 16, 268.

Mac Callum, W G. (1902), Die Beziehungen der Lymphgefäße zum Bindegewebe. Arch Anat. u. Physiol Anat Abt 273.

Mac Callum, W. G. (1903), On the mechanism of absorption of granular materials from the peritoneum. Bull. John Hopkins Hosp. 14, 105.

McCarrell, J. D (1939a), Lymphatic absorption from the nasopharynx. Am. J. Physiol. 126, 20

McCarrell, J D (1939b), Cervical lymph pressure in the dog Am. J. Physiol 127, 154.

McCarrell, J. D. (1939/40), The effect of warm and cold nasopharyngeal irrigation on cervical lymph flow Am. J. Physiol. 128, 349.

McCarrell, J. D. (1940), The effects of hyperthermia and hypothermia on cervical lymph flow. Am. J Physiol. 130, 34

McCarrell, J. D & C. K. Drinker (1941), Cervical lymph production during histamine shock in the dog Am J. Physiol 133, 64

McCarrell, J. D, S Thayer & C K Drinker (1941), The lymph drainage of the gall bladder together with observations on the composition of liver lymph Am. J. Physiol. 133, 79

[illegible]

McClean, D (1942), The in vivo decapsulation of [illegible] by hyaluronidase. J Path. Bacter. 54, 284.

McClean, D (1943), Methods of assay of hyaluronidase and their correlation with skin diffusing activity Biochem J 37, 169

McClure, C [illegible]
Tr internat C [illegible]

McClure, [illegible]
intradermally [illegible]

Mc Gillav [illegible]
Math -naturw Cl. Wien I [illegible]

Mac Kay, E M, M D Jordan & L L Mac Kay (1949), Experimental pulmo- [illegible] edema caused by ammonium ion.

[illegible] hkoff & R E Hyatt (1949), Experi- [illegible] 529

[illegible] Passage of radio-active erythrocytes from the peritoneal cavity into the blood stream during experimental ascites. J Exp Med 91, 599

McKee, F. W, W. G Wilt Jr., R. E. Hyatt & G. H. Whipple (1950), The circulation of ascites fluid. Interchange of plasma and ascites fluid protein as studies by means of $C^{14}$ labelled lysine in dogs with constriction of the vena cava. J Exp. Med 91, 115

Lewis, T. [illegible] one of the minute vessels of the [illegible] of measurement of pressure wi[illegible]

Leydhecker, O. (1893), Ueber einen fall von Carcinom des Ductus thoracicus mit chylösem Ascites. Virchows Arch. 134, 118.

Lichtman, S. S. (1949), Diseases of the liver, gall bladder and bile ducts Lea & Febiger, Philadelphia.

Lieben, S. (1910), Über die Fortbewegung der Lymphe in den Lymphgefäßen. Zentralbl. Physiol. 24, 1164

Liebermeister, G. (1922), Starke Erweiterung des Sinus coronarius cordis mit Insuffizienz der Valvulae Thebesii bei relativer Tricuspidalinsuffizienz Klin. Wochenschr. 1, 1463

Liebesny, P (1923), Untersuchungen über die Capillarisdruckmessung Pflugers Arch. 198, 215.

Lilienfield, L. S., E. D. Freis, E A. Partenope & H. J. Morowitz (1955), Transcapillary migration of heavy water and thyocyanate ion in the pulmonary circulation of normal subjects and patients with congestive heart failure. J Clin. Investig. 34, 1.

Lindemann, W. (1899), Ueber Veränderungen der Nieren infolge von Ureterenunterbindung. Z Klin Med 34, 299.

Lindner, E., W. Marx & H. E. Kruger (1944), Absence in lymph of capillary permeability factors in traumatic shock. Proc Soc. Exp Biol Med 55, 181.

[illegible] Restimmung [illegible] 104, 470

[illegible] oids. Am. J. Physiol 134, [illegible]

Loeschke, H. (1934), Experimentelle Untersuchungen über Saftstrom- und Resorptionswege. Virchows Arch 292, 281

Long, C. N. H. & E. G. Fry (1945), Effect of epinephrine on adrenal cholesterol and ascorbic acid. Proc. Soc. Exper. Biol. & Med. 59, 67.

*Lorber, V. (1940), Lymph flow from the heart-lung preparation during pulmonary edema* Proc Soc. Exp Biol Med. 43, 170

Lowenberg, E. L. (1952), Edema and lymphedema of the lower extremities Virg Med. Monthly. 79, 351.

Lowgren, E (1952), Lymphuria as an explanation of the postural proteinuria Acta Med. Scand. 144, 245.

Lowgren, E. (1955), Studies on benign proteinuria, with special reference to the renal lymphatic system Acta Med. Scand Suppl. 300.

Lubsen, N (1936), Les coeurs lymphatiques chez la grenouille. Arch. Nederl de Physiol. 21, 587.

Luciani's (1911), Human Physiology. Vol. 1 MacMillan, London.

Lucke, B. (1944), The pathology of fatal epidemic hepatitis Amer. J Path 20, 471.

Ludány, Gy. (1929), Das Elektrogramm des Lymphherzen des Frosches Arbeiten der II (Medizinisch-naturwissenschaftlichen) Abteilung der Wissenschaftlichen Stefan Tisza Gesellschaft in Debrecen, 3, 317.

Ludwig, C. (1858), Lehrbuch der Physiologie des Menschen. Leipzig und Heidelberg.

Ludwig, C. & [illegible] der Niere des Säugetieres. Sitz.-Ber. [illegible]

Ludwig, K. [illegible] gefäße im Hoden. Sitz.-Ber Wien. [illegible]

Luft, R., B. Sjögren, O. Cassmer & E. Issen (1950), The effect of intravenous administration of adrenaline and dl-nor-adrenaline on the circulating eosinophils and lymphocytes. Acta Endocrinol 4, 153.

Luhan, J. A. (1936), Hemiedema in cases of hemiplegia. Arch Neurol Psychiatr. 36, 42.

Luisada, A. (1926), Un nuovo tonometro capillare; la pressione capillare nell'uomo normale. Cuore e circolaz. Roma 10, 55.

Luisada, A. A. (1940), The pathogenesis of paroxysmal pulmonary edema. Medicine 19, 475.

Luisada, A. A. (1943), The treatment of paroxysmal pulmonary edema, with special reference to formes occurring under wartime conditions. Exp Med. & Surg. 1, 22.

Luisada, A. A. & S. J. Sarnoff (1944), Studies of paroxysmal pulmonary edema. A new experimental method. Proc. Soc. Exp. Biol Med. 57, 279.

Luisada, A. A. & S. J. Sarnoff (1946), Paroxysmal pulmonary edema consequent to stimulation of cardiovascular receptors. II. Effect of intra-arterial and intravenous infusions. Am. Heart J. 31, 270.

Lurie, M. B. (1959), Mechanisms affecting spread in tuberculosis Ann. New York. Acad. Sc. 52, 1074.

Macallum, A. B (1891/92), On the demonstration of the presence of iron chromatin by microchemical methods. Proc. Roy. Soc. 50, 277

Macallum, A. B. (1894), On the absorption of iron in the animal body J. Physiol 16, 268.

Mac Callum, W. G. (1902), Die Beziehungen der Lymphgefäße zum Bindegewebe. Arch Anat. u. Physiol. Anat. Abt. 273.

Mac Callum, W. G. (1903), On the mechanism of absorption of granular materials from the peritoneum. Bull. John Hopkins Hosp. 14, 105.

McCarrell, J. D. (1939a), Lymphatic absorption from the nasopharynx. Am J. Physiol. 126, 20.

McCarrell, J. D (1939b), Cervical lymph pressure in the dog. Am J. Physiol 127, 154

McCarrell, J. D. (1939/40), The effect of warm and cold nasopharyngeal irrigation on cervical lymph flow. Am. J. Physiol. 128, 349

McCarrell, J. D (1940), The effects of hyperthermia and hypothermia on cervical lymph flow Am J Physiol 130, 34

McCarrell, J. D. & C. K. Drinker (1941), Cervical lymph production during histamine shock in the dog. Am J Physiol. 133, 64

McCarrell, J. D., S Thayer & C K. Drinker (1941), The lymph drainage of [illegible] lymph Am. [illegible]

[illegible] genic bacteria [illegible] 47

[illegible] e activity of testicular extracts, bacterial culture filtrates and other agents that increase tissue permeability. Biochem J 35, 159.

McClean, D (1942), The in vivo decapsulation of streptococci by hyaluronidase. J. Path. Bacter 54, 284

McClean, D (1943), Methods of assay of hyaluronidase and their correlation with skin diffusing activity Biochem J. 37, 169

McClure, C F. W, (1914), The development of the lymphatic system in fishes. Tr internat Cong Med I Anat & Embryol 231, London

McClure, W B & C. A Aldrich (1923), Time required for disappearance of intradermally injected salt solution J Am. Med Ass 81, 293

Mc Gillavry, T H (1864), Zur Anatomie der Leber Sitzungsb Akad. Wiss. Math.-naturw Cl. Wien I

Mac Kay, E M, M D Jordan & L L Mac Kay (1949), Experimental pulmonary edema II Pathogenesis of pulmonary edema caused by ammonium ion. Proc Soc Exp Biol Med 72, 421.

McKee, F. W, J A Schilling, G H Tishkoff & R E. Hyatt (1949), Experimental ascites Surg, Gynec & Obstetr. 89, 529.

McKee, F W. & W. B Stewart (1950), Passage of radio-active erythrocytes from the peritoneal cavity into the blood stream during experimental ascites. J. Exp. Med 91, 599

McKee, F. W, W. G. Wilt Jr, R. E Hyatt & G. H. Whipple (1950), The circulation of ascites fluid Interchange of plasma and ascites fluid protein as studies by means of $C^{14}$ labelled lysine in dogs with constriction of the vena cava. J Exp Med 91, 115

McKee, F. W., C. L. Yuile, B. G. Lauson & G H. Whipple (1952), Albumin and globulin circulation *in experimental* ascites Relative rates of interchange between plasma and ascites fluid studied with $C^{14}$ labelled proteins. J. Exp. Med. 95, 161

MackMull, G. & N. A. Michels (1932), Absorption of colloidal carbon from the peritoneal cavity in the teleost. Tautogolabrus adspersus. Am. J. Anat. 51, 3.

McMaster, Ph. D. (1937a), *Changes in* the cutaneous lymphatics of human beings and in the lymph flow under normal and pathological conditions. J. Exp Med. 65, 347.

McMaster, Ph. D. (1937b), The lymphatics and lymph flow in the edematous skin of human beings with cardiac and renal disease. *J. Exp. Med 65, 373.*

McMaster, Ph. D. (1941a), Intermittent take-up of fluid from the cutaneous tissue J. Exper. Med. 73, 67.

McMaster, Ph. D. (1941b), Factors influencing the intermittent passage of Locke's solution into living skin. J. Exper. Med. 73, 85.

McMaster, Ph D. (1942), Lymphatic participation in cutaneous phenomena (Harwey Lecture). Bull. New York. Acad Med. 18, 731.

McMaster, Ph. D. (1946a), The pressure and interstitial resistance prevailing in the normal and edematous skin of animals and man. J. Exp Med. 84, 473.

McMaster, Ph. D. (1946b), The effects of venous obstruction upon interstitial pressure in animal and human skin. J. Exp. Med. 84, 495.

McMaster, Ph D. (1947), The relative pressure within cutaneous lymphatic capillaries and the tissues J. Exp Med. 86, 293.

Mc Master, Ph. D & S. Hudack (1932), Induced alterations in the permeability of the lymphatic capillary. J. Exp. Med 56, 239.

McMaster, Ph. D. & S. S. Hudack (1935), The formation of agglutinins within lymph nodes. J. Exp. Med 61, 783.

[illegible] spread of s[illegible]

[illegible] existing in co[illegible] state of the fluid in the intradermal tissue J Exp. Med. 69, 247

McMaster, Ph D & R. J. Parsons (1950), The movement of substances and the state of the fluid in the intradermal tissue Annals N. Y. Acad. Sci. 52, 992.

[illegible] and capillary permeabili[illegible]

[illegible] in menschlichen und tierischen Geweben, ihr Verhalten in serösen Häuten und ihre Bedeutung fur deren Pathologie. Dtsch Ztschr. Chir 175, 147.

Magnus, R. (1899), Über die Entstehung der Hautodeme bei experimenteller hydrämischer Plethora. Arch. exp. Med. 42, 250.

Makino, J. (1924), Beiträge zur Frage der hepatocellulären Gallenfarbstoffbildung Zieglers Beitr 72, 808.

Mall, F. P. (1901), On the origin of the lymphatics in the liver Bull. J. Hopk Hosp 12, 146.

Mallet-Guy, P, G Devic, J Feroldi & P. Desjacques (1954), Experimental study of ascites: posthepatic venous stenosis and transposition of liver into thorax. Lyon. chir. 49, 153.

Maloet (1752), Clinical description.

(Mamiyev, S. [illegible] века. Труды Лени[illegible]

Mancke, R. & [illegible] und der Hepatitis [illegible]

Mann, E. C. (1914), The peripheral origin of surgical shock. Bull. John Hopkins Hosp. 25, 205.

Mann, J. O., F. O. Mann & J. L Bollman (1949), Hypoprothrombinaemia due to loss of intestinal lymph. Am. J Physiol. 158, 311

Mansfeld, G. (1948), Az infectio és immunitás élettana (Physiology of infection and immunity). Orvosok Lapja (Hungarian), 4, 321.

Manwaring, W. H., R. E. Monaco & H. P. Marino (1923), Hepatic reactions in anaphylaxis. VI. Histamine reactions in isolated canine tissues. J. Immunol 8, 217.

Manwaring, W. H. and others (1927), Relations of anaphylaxis to immunity; normal and anaphylactic detoxication of specific foreign proteins. J. Immunol 13, 357.

Marchand, P. (1951), Anatomy and applied anatomy of mediastinal fascia. Thorax 6, 359.

Markowitz, C. & F. C. Mann (1931), Studies on the physiology of the liver. XXI. The role of the liver in the formation of lymph. Am J. Physiol 96, 709.

Martin, G. J. & J. M. Beiler (1952), Effect of phosphorylated hesperidin, a hyaluronidase inhibitor, on fertility in the rat. Science 115, 402

Martin, G. J. (1953), Phosphorylated hesperidin. Science 117, 363.

Martin, H. E. (1932), Physiological leukocytosis. The variation in the leukocyte count during rest and exercise and after the hypodermic injection of adrenaline. J. Physiol. 75, 113

Martin, S. (1890), Details of a case of chylous ascites and chylous hydrothorax. Lancet 2, 875.

Mascagni, P. (1787), Vasorum lymphaticorum corporis humani descriptio et iconographia. Siena.

Maschmann, E. (1938), Über Bakterienproteasen. IX Die Anaerobiase Biochem. Zeitschr. 297, 284

Mathews, M. B. & A. Dorfman (1954), Inhibition of hyaluronidase In· Asboe—Hansen.

(Matotchkin, I. N.) Матючкин, И И (1949), Лимфатические сосуды диафрагмы и ее всасывающая способность Об трудов Архангельск мед ин-та 9, 89

Maung Kyaw Myint (1957), Some functions of the renal lymphatics. Experimental observations Burma Medical Journal. 5, 13.

Maurer, F. W. (1938), Isolation and analysis of extracellular muscle fluid from frog. Am. J. Physiol 124, 546

Maurer, F. W. (1940), The effects of decreased blood oxygen and increased blood carbon dioxide on the flow and composition of cervical and cardiac lymph. Am. J. Physiol 131, 331

Maurer, F. W (1941), The effects of carbon monoxide anoxaemia on the flow and composition of cervical lymph Am J Physiol 133, 170

Maurer, F. W., M F Warren & C. K. Drinker (1940), The composition of mammalian pericardial and peritoneal fluids Am J Physiol 129, 635.

Mautner, H (1924), Die Bedeutung der Venen und deren Sperrvorrichtung für den Wasserhaushalt Wien Arch inn Med 7, 251

Mautner, H & E P. Pick (1915), Ueber die durch »Shockgifte« erzeugten Zirkulationsstörungen Münch. med Wschr. 62, 1141

Mautner, H & E P Pick (1922), Über die durch Shockgifte erzeugten Zirkulationsstörungen II Das Verhalten der überlebenden Leber Biochem Ztschr. 127, 72

Mautner, H & E. P. Pick (1929), Über die durch Shockgifte erzeugten Zirkulationsveränderungen, der Einfluß der Leber auf Blutdruck und Schlagvolumen Arch exper Path Pharmakol 142, 271

Maximov, A (1927), Bindegewebe und blutbildende Gewebe In· Mollendorffs Handb d Hist Bd II Springer, Berlin

Maximov, A A & W Bloom (1939), Textbook of histology Saunders & Co, Philadelphia—London

Mayer, R L & F C. Kull (1947), Influence of Pirybenzamine and Antistine upon the Action of Hyaluronidase Proc Soc Exp Biol Med. 66, 392.

Mayer, S (1886), Studien zur Histologie und Physiologie des Blutgefäßsystems. Gerold's Sohn, Wien

Mayo, C. Jr & C H Greene (1929), Studies in metabolism of bile; role of lymphatics in early stages of development of obstructive jaundice. Am. J Physiol. 89, 280.

Meckel (1772), Dissertatio epistolaris de vasis lymphaticis glandulisque conglobat ad Haller Berolini

Meier, R., F. Gross & P. Desaulles (1951), Über die Bedeutung der Nebenniere und der Nebennierenrindensteroide fur den Ablauf entzundlicher Reaktionen. Klin. Wschr. 29, 653.

Meigs, J. V. (1954), Fibroma of the ovary with ascites and hydrothorax, Meigs' syndrome Am. J. Obstet Gynec. 67, 962.

Meigs, J. V. & J. W. Cass (1937), Fibroma of ovary with ascites and hydrothorax with report of 7 cases Am J. Obstet. Gynec. 33, 249.

Meigs' syndrome (1954). Lancet 2, 231.

Melli, G & M. Pisa (1931), Ricerche sperimentali sull edema polmonare acuto. Minerva Medica 2, 419.

Meltzer, S. J & C. Meltzer (1903), On difference in the influence upon inflammation between the section of the sympathetic nerve and the removal of the sympathetic ganglion J. Med. Research 10, 135.

(Melnikov, A V.) *Мельников, А. В.* (1945), О раке желудка (Клинический очерк.) Ленинград

Mende, F. (1919), Über Hyperämie und Ödem bei der Hemmung des Ruckflusses des venosen Blutes durch die Staubinde. Dtsch. Z. Chir. 150, 379.

Mendel, L. B (1895/96), On the passage of sodium iodide from the blood to the lymph, with some remarks on the theory of lymph formation J. Physiol. 19, 217.

Meneely, G. R, G. Qualls & H. J Curtis (1951), Rate of uptake of fluid from alveolus measured with radio-isotopes; comparison of rapid rate for water with slower rate for saline. Am J. Physiol. 167, 809.

[illegible] from the [illegible] in- [illegible]

Menkin, V (1930a), The accumulation of iron in tuberculous areas. Proc. Soc Exp. Biol. Med. 27, 1020.

Menkin, V (1939b), Studies of inflammation. III. Fixation of metal in inflamed areas J. Exp Med 51, 879.

Menkin, V (1931a), Studies on inflammation. V. The mechanism of fixation by the inflammatory reaction. J. Exp. Med. 53, 171.

Menkin, V. (1931b), Studies of inflammation VI Fixation of trypan blue in inflamed areas of frog. J. Exp. Med 53, 179

Menkin, V. (1931c), Studies of inflammation. VII Fixation of bacteria and of particulate matter at the site of inflammation. J. Exp Med. 53, 647.

Menkin, V (1931d), Aspect of inflammation in relation to immunity. Arch. Path. 12, 802.

Menkin, V (1936), Studies of inflammation XII. Mechanism of increased [illegible] J. Exp. Med. 64, 485. [illegible] con- [illegible] uko- [illegible]

Menkin, V. (1940a), Effect of adrenal cortex extract on capillary permeability Am J. Physiol 129, 691

[illegible] the mechan- [illegible]

Anat. u Physiol. Physiol. Abt. [illegible]

Merkel, F. (1909), Betrachtungen über die Entwicklung des Bindegewebes. Anat H. 38, 321.

[illegible] V. (1879) Recherches sur lymphatiques de la couche sous-séreuse [illegible]

[illegible] morrák diagnosztikájában (Errors in the [illegible] (Hungarian), 93, (46). 1317

[illegible] Nakazawa (1928), Oedeme und herab- [illegible] ser Anämie. Die Plasmacolloide und der colloidosmotische Druck im Plasma. Acta Med Scand Suppl. 26, 218.

Meurers, K. (1925), Über die Folgen der gleichzeitigen oder ungleichzeitigen Unterbindung der Carotides communes bei Kaninchen. Ztschr ges. exp. Med. 46, 135

Meyer, A. W. (1906), An experimental study on the recurrence of lymphatic glands and the regeneration of lymphatic vessels in the dog Bull. John Hopkins Hosp 17, 185

Meyer, E. & R. Meyer-Bisch (1924), Weitere Mitteilung uber die Pathogenese des Diabetes insipidus. Klin. Wschr. 3, 1796

Meyer, F. & G. Holland (1933), Die Messung des Druckes in den Geweben I Der Gewebsdruck beim Ödem. III. Arch exper Path Pharmakol 168, 380

Meyer, K. (1947), The biological significance of hyaluronic acid and hyaluronidase. Physiol. Reviews 27, 335.

Meyer, K. & E. Chaffee (1941), The mucopolysaccharides of skin. J. Biol Chem 138, 491.

Meyer, K., E. Chaffee, G. L. Hobby & M H. Dawson (1941) Hyaluronidase of bacterial and animal origin. J Exp Med. 73, 309

Meyer-Bisch, R. & F Günther (1926), Physiologie und Pathologie der Lymphbildung. Ergebn. Physiol. 25, 574

Meyer-Bisch, R., F. Günther & D Bock (1925), Untersuchungen an der Brustganglymphe des Hundes 5. Mitt Über den Einfluß des Insulins und Adrenalins auf die Lymphe Pflügers Arch 211, 341

Mihalkovics, V. (1873), Beiträge zur Anatomie und Histologie des Hodens Ber sächs Ges. Wiss Math -phys Cl

Miles, A. A , D. L. Wilhelm, (1955), Enzyme-like globulins from serum reproducing vascular phenomena of inflammation, activable permeability factor and its inhibitor in guinea-pig serum Brit J. Exp Path 36, 71.

Miles, A. A , D. L. Wilhelm (1958), Distribution of globulin permeability factor and its inhibitor in the tissue fluid and lymph of the guinea pig Nature, 181, 96.

Miller, L. L., W F Bale, C L. Yuile, R E. Masters, G H Tishkoff & G H. Whipple (1949), Use of radio-active lysine in studies of protein metabolism Synthesis and utilisation of plasma proteins J. Exper Med 90, 297.

Miller, R. G (1938), The influence of inflammation on the absorption of substances of varied diffusibility J. Exp Med. 67, 619

Miller, W. H & A M Dessert (1949), The inhibition of hyaluronidase by alphatocopheryl esters Am New York. Acad. Sc. 52, 167.

Miller, W. S. (1900), Das Lungenläppchen, seine Blut- und Lymphgefäße Arch. Anat. u. Physiol. Anat Abt 179.

Miller, W. S (1947), The lung. C. C Thomas, Springfield, Illinois.

Milroy, W. F. (1928), Chronic hereditary edema, Milroy's disease. J A. M. A. 91, 1172

(Minkina, N. A.) *Минкина, Н. А* (1953). Лимфатическая система реберной и диафрагмальной плевры человека Труды Ленингр. сан.-гиг. мед. ин-та 17, 47

Minkowski, O. (1892), Diskussion über Lebercirrhose. Verh. XI. Kongr. inn Med 127.

Minkowski, O. (1904), Zur Pathogenese des Ikterus. Zeitschr. Klin. Med. 55, 34.

Modrakowski, M. G. (1914), Beobachtungen an der überlebenden Säugetierlunge. II. Über die experimentelle Erzeugung von Lungenödem. Pflügers Arch. 158, 527.

Moers, A. (1864), Über den feineren Bau der Nebenniere. Virchows. Arch. 29, 340.

(Mogilnitski, B. N.) *Могилницкий Б. Н.* (1949), Вопросы проницаемости кровеносных капилляров в патологии. Медгиз, Москва.

Mollendorff, W. (1939), *Handb. d Mikr. Anat. d. Menschen.* Springer, Berlin.

Moon, V. H. (1938), Shock and related capillary phenomena. Oxford University Press, New York.

Moon, V. H. (1942), Shock. Its dynamics, occurrence and management. Lea & Febiger, Philadelphia

Moore, A. (1901), *The effects of ions on the contraction of the lymph hearts of frogs.* Am. J Physiol. 5, 87.

Moore, A. (1901), Are the contractions of lymph hearts of the frog dependent upon centres situated in the spinal cord? Am J. Physiol. *5*, 196.

Moore, D H. (1953), Electrophoretic study of tissue extracts and sera of mice after shock-producing injuries. *Am. J. Physiol* 173, 131

Moore, D. H., J. L. Nickerson, A E. Powel & G. Marks (1951), A study of the transfer of serum proteins into tissue injured by tourniquet. Proc. Soc. Exp. Biol. Med. 77, 706

Morel, F. & M. Marois (1948), La perméabilité capillaire au sodium mesurée chez le lapin à l'aide du radio-sodium. *C. R. Soc. Biol.* 142, 1366.

Morel, F. & M Marois (1949), Méthode de mesure du taux des échanges de sodium entre le plasma et les liquides extravasculaires chez le lapin à l'aide du radiosodium. Arch. Sci. Physiol. 3, 15.

Morner, K. A. H. (1895), Untersuchungen uber die *Proteinstoffe* und die eiweißfällenden Substanzen des normalen Menschenharns. Scand Arch. Physiologie 6, 332.

Morris, B (1953), The effect of diaphragmatic movement on the absorption of protein and red cells from the peritoneal cavity. Austral J. Exp. Biol. Med. Sci. 31, 236.

Mortensen, O. A. & W. E. Sullivan (1933), The cerebrospinal fluid and the cervical lymph nodes Anat Rec. 56, 359.

Morton, R. (1689), Phythysiologia seu exercitationes de phthysi Lib I. Smith, London

Most, A. (1899), Über die Lymphgefäße und die regionären Lymphdrüsen des Magens in Rucksicht auf die Verbreitung des Magencarcinoms. Langenbecks Arch 59, 175

Most, A. (1917), Neue deutsche Chirurgie *Bd* Chirurgie der Lymphgefäße und der Lymphdrusen. Enke, Stuttgart

Muller, L R (1896), Beiträge zur Histologie der normalen und der erkrankten Schilddruse. Zieglers Beiträge 19, 127.

Muller, E F. & W. F. Petersen *(1926)*, *Splanchno-peripheres Gleichgewicht* und Gefäßpermeabilität. Klin. Wschr 5, 53.

Munk, I (1884), Zur Lehre von der Resorption, Bildung und Ablagerung der Fette im Tierkorper Virchows Arch. 95, 407.

Munk, I. (1895), Zur Kenntnis der interstitiellen Resorption wasserloslicher *Substanzen Dubois Archiv.* 387.

Munk, I. & H. Friedenthal (1901), Ueber die Resorption der Nahrungsfette und den wechselnden Fettgehalt des Blutes nach Unterbindung des Ductus thoracicus Zentralbl Physiol 15, 297.

Munk, I. & A Rosenstein (1891a), Zur Lehre von der Resorption im Darm, nach Untersuchungen einer Lymph-(Chylus-)fistel beim Menschen. Virchows Arch 123, 230.

Munk, I. & A. Rosenstein (1891b), Zur Lehre von der Resorption im Darm nach Untersuchungen an einer Lymph-(Chylus-)fistel beim Menschen. Virchows Arch. 123, 484

Muscatello, G. (1895), Über den Bau und das Aufsaugungsvermogen des Peritoneums. Virchows Arch. 142, 327.

Myers, J D. & W ... venous pressure by occlusive catheteriz ... g. 30, 662 (P).

Mylon, E., M. C. ... ies on therapy in traumatic shock. Ar

Mylon, E., M. C. Winternitz, R. Katzenstein & G. J. Sütö-Nagy (1942), Studies on mechanism involved in shock. Am. J. Physiol. 137, 280

(Nadezhdin, V. N.) *Надеждин, В. Н.* (1951), Архитектура начальных лимфатических сетей кожи нижней конечности человека. Труды Ленингр. сан.-гиг. мед. ин-та 9, 115.

Nageotte, J. (1916), Essai sur la nature et la genèse des substances conjonctives. C. R. Soc. Biol. 79, 1121.

Nagy, I. & L. Sós (1951), Megjegyzések a Disse-tér kérdéséhez (Comments upon the question of Disse's space). Kísérl. Orvostud. (Hungarian), 3, 170

Nakahara, W. & J. B. Murphy (1921), On the nature of the so-called germ center in lymphoid tissue. Anat. Rec. 22, 107.

(Nalintzev, M. E.) *Налинцев, М. Е.* (1951), Лимфатические сосуды кожи туловища человека. Труды Ленингр. сан.-гиг. мед. ин-та 9, 97

Natucci, G. & L. Giarelli (1951). Sull'importanza della linfostasi nella patologia splenica. Riv. anat. pat. e onc. 4, X.

Netsky, N. G. & S. S. Leiter (1943), Capillary permeability to horse proteins in burn-shock. Am. J. Physiol. 140, 1.

Neuenkirchen (1890), Ein Fall von Chylothorax. St. Petersburger Med. Wschr. 7, 459.

Neuman, J. (1873), Zur Kenntnis der Lymphgefäße der Haut des Menschen und der Säugetiere. Wien.

Newton, W. H. (1932), Pulmonary edema in the cat heart-lung preparation. J. Physiol. 75, 288.

Nicolesco, J. (1930), Sur les lymphatiques du rein. Ann. d'anat. path. 7, 503

Nikolski (1895), Zur Frage über Bluteingießungen in die Bauchhöhle. Inaug.-Diss. Petersburg. cit. Orlov.

Nix, J. T., E. F. Flock & J. L. Bollman (1951), Influence of cirrhosis on proteins of cysternal lymph. Am. J. Physiol. 164, 117

Nix, J. T., F. C. Mann, J. L. Bollman, J. H. Grindlay & E. W. Flock (1951), Alteration of protein constituents of lymph by specific injury to liver. Am. J. Physiol. 164, 119.

Noble, J. W. (1958), Chylothorax. An unusual complication of left heart catheterization. Bull. St. Francis Hosp. and Sanat. 15, 22

Nolf, P. (1905/06), L'action lymphagogue de la propeptone. Arch. internat. Physiol. 3, 229.

(Norenberg-Ticharkvia ... (1934), Острый аппендицит в свете совре... 5/22.

Notkin, J. A. (1925), ... Bedeutung der Lymphgefäße.) Virchows Arch. 225, 4...

Nuck, A. (1692), Adenographia curiosa et uteri foeminei anatome nova. Lugd. Batav.

Nuhn, A. (1878), Lehrbuch der vergleichenden Anatomie. Bd. 1. Heidelberg

Oakley, C. L., G. H. Warrack & W. E. Van Heyningen (1946), The collagenase (Kappa-toxin) of Cl. welchii type. A. J. Path. Bact. 58, 229

Oberndorfer, S. (1925), Atherosklerose des Ductus thoracicus. Verh. deutsch. Pathol. Ges. 20. Tagung 247.

Ochsner, A. & M. De Bakey (1940), Therapy of phlebothrombosis and thrombophlebitis. Arch. Surgery 40, 208.

Ochsner, A. & M. De Bakey (1949), Postphlebitic sequelae. J. A. M. A. 139, 423

Oeff, K. (1954 a), Umsatz von radioaktiven Serumeiweißreaktionen. II. Versuche an normalen Ratten. Z. ges. exp. Med. 123, 309.

Oeff, K. (1954 b), Umsatz von radioaktiven Serumeiweißfraktionen. III. Versuche an normalen Kaninchen. Ztschr. ges. exp. Med. 123, 434.

Oehme, C ... und pathologischen Ph...

Ogata, L ... sons du syst...

C. R. Soc. Biol. 130, 1502.

Ogata, T (1913), Beitrage zur experimentell erzeugten Lebercirrhose und zur Pathogenese des Ikterus mit spezieller Berucksichtigung der Gallenkapillaren bei der Unterbindung des Ductus choledochus und der Ikterogenvergiftung. Zieglers Beitrage 55, 236.

(Okun, N S.) *Окунь Н С* (1943), Лимфообращение при инфекции Вест хип 63, 27

Okuneff, N. (1924), Über den Einfluß lokaler thermischer Reize auf die Abwanderung *eines intravenos injizierten kolloidalen Farbstoffs aus dem Blute* Pflugers Arch. 204, 261.

Okuneff, N (1926), Über die vitale Farbstoffimbibition der Aortenwand Virchows Arch 259, 685.

(Okuneff, N. V.) *Окунев, Н В* (1939), Проблема проницаемости в патологии Проблемы проницаемости Москва

(Oleandrov, L V.) *Олеандров, Л. В* (1939), Патологические изменения проницаемости Проблемы проницаемости Москва

Oliver, J. *(1950)*, *The morphological aspects of renal tubular secretion* and reabsorption Renal Function Transaction of the 1st Conference. J. Macy Found 1949. New York.

Oliver, J, T Bloom & C N. Mangieri (1947), On origin of heparin; examination of heparin content and specific cytoplasmic particles of neoplastic mast cells J. Exper. Med. 86, 107.

Olivo, O M (1933), Organismo e tessuti. Minerva med. 1, 321.

Omodei-Zorini, A. (1928), Sulla «splenogranulomatosi siderotico» di Gamna e le cosidette «micosisplenіche» degli autori francesi Arch pat e clin Med 7, 121.

Opie, E L. (1924), The fate of antigen (protein) in an animal immunized against it. J. Exp Med. 39, 659.

Opie, E L. (1929), Inflammation and immunity. J Immunol 17, 329

Opie, E. L. & M. B Rothbard (1953a), Osmotic homeostasis maintained by mammalian liver, kidney and other tissues. J. Exp. Med. 97, 483.

Opie, E L & M B Rothbard (1953b), Water exchange of collagenous tissue and of gelatin J. Exp Med 97, 499

Opsahl, J. C. (1949a), Dermal spreading of India ink with and without hyalu-
s from adrenal cortex. Yale J. Biol Med 21, 487.
f certain steroids in adrenal-hyaluronidase rela-
115.
& E. G. Fry (1951), Chorionic gonadotrophin,
relationship Yale J. Biol. Med 23, 391
Versuche uber die Resorption in der Bauchhoble Pflugers Arch. 59, 170.

Orts Llorca, F (1931), Lymphatiques du sternum, de la clavicule et des corps vertebraux Ann d'anat. path 8, 769

Orts Llorca, F. & J. Botár (1932), Lymphatiques des ganglions de la chaine sympathique chez le nouveau-né. Ann d'anat path 9, 818.

Orts Llorca, F & J. Botár (1933), Collecteurs lymphatiques de la prostate. Ann. d'anat path 10, 37

Osato, S. (1921/22), Beiträge zum Studium der Lymphe 2 Mitt. Übergang der verschiedenen, in die *Blutbahn eingespritzten Substanzen vom Blut in die Lymphe.* Tohoku J. exper Med 2, 465

Ostrowski, W (1935), Badania doswiadczalne nad szetaczianiem krwi do jamy oplucnej. Acad Polon sc et. lett. Cl med 10, 819.

Ottaviani, G. (1947), Peut-on parler de lymphocrinie hypophysaire? Bull. d'Histiol Appl 146

Ottaviani, G (1949), Osservatione sull'importanza anatomofunzionale dei vasi linfatici della tiroire. Non Zool Ital. 68: Suppl Atti Soc. Ital Anat. XI Conv. Catania.

Ottaviani, G. (1951), Ricerche istologiche sulla ghiandola tiroidea in stasi linfatica sperimentale. Folia Endocrinologica 4, 19

Ottaviani, G. (1955), In L'Ateneo parmense. Vol XXVI Suppl

Ottaviani, G. & M. Cavalli (1933). Effetti della estirpatione dei linfonodi del collo nel cane. Atti della Soc. med.-chir. di Padova, 661

Ottaviani, G., L. Frignani, B. Cenna (1957). Il sistema linfatico azygos. L'Ateneo Parmense, 28: 71, suppl. 4.

Paine, R. B., H. R. Butcher, F. A. Howard & J. R. Smith (1949a). Observations on mechanisms of edema formation in the lung. J. Lab. & Clin. Med. 34, 1544.

Paine, R. B., H. R. Butcher, F. A. Howard & J. R. Smith (1949b). A technique for the collection of lymph from the right thoracic duct in dogs. J. Lab. & Clin. Med. 34, 1576

Paine, R., H. R. Butcher, J. R. Smith & F. A. Howard (1950). Observations on the role of pulmonary congestion in the production of edema of the lungs. J. Lab. & Clin. Med. 36, 288

Paine, R., J. R. Smith, H. R. Butcher & F. A. Howard (1952). Heart failure and pulmonary edema produced by certain neurologic stimuli. Circulation 5, 759

*Palay*, S. L. & K. J. Karlin (1959a). An electron microscopic study of the intestinal villus. I. The fasting animal. J. Bioph. Bioch. Cytol. 5, 363

Palay, S. L. & L. J. Karlin (1959 b). An electron microscopic study of the intestinal villus. II. The pathway of fat absorption. J. Bioph. Bioch. Cytol. 5, 373

Panizza, B. (1833a). Osservazioni antropo-zootomico fisiologiche. Pavia

Panizza, B. (1833b). Sopra il sistema linfatico dei rettili. Pavia.

Panthitschko, M. & E. Kaiser (1951). Untersuchungen über Aktivierung und Hemmung der Hyaluronidase. Biochem. Ztschr. 322, 137

Papilian, V. & G. Russu (1940). Căile limfatice colaterale din torace la câine. Cluj med. 21, 74

Papp, M. (1955). Personal communication.

Papp, M. & I. Fodor (1957a). Vizsgálatok a nyálmirigyek nyirokérrendszeréről kutyában (Study on the lymph vascular system of the salivary glands in the dog) (Hungarian)

Papp, M. & Fodor (1957b). Vizsgálatok a lép periarteriális „résekről" kutyában (Study on the periarterial "clefts" in the spleen of the dog) (Hungarian)

Papp, M., E. Németh, I. Feuer & I. Fodor. (1957). A nyirokkeringés akadályozottságának hatása a kísérletes acut pancreatitisre (Effect of lymphatic obstruction on experimental acute pancreatitis). Orv. Hetil. (Hungarian), 580

Papp M., E. Zádory, F. Soltı, I. Holló (1957). Über die elektrischen Erscheinungen der Funktion der Froschlymphherzen. Acta Physiol. Acad. Sci. Hung. 12, 153.

Pappenheimer, J. R. (1953). *Passage of molecules through capillary walls.* Physiol. Reviews 33, 387

Pappenheimer, J. R. & W. B. Kinter (1956). Hematocrit ratio of blood within mammalian kidney and its significance for renal hemodynamics. Am. J. Physiol. 185, 377.

Pappenheimer, J. R. & A. Soto-Rivera (1948). Effective osmotic pressure of plasma proteins and other quantities associated with the capillary circulation in the hind limbs *of cats and dogs*. Am. J. Physiol. 152, 471

Pappenheimer, J. R., E. M. Renkin & L. M. Borrero (1951). Filtration, diffusion and molecular sieving through peripheral capillary membranes; contribution to pore theory of capillary permeability. Am. J. Physiol. 137, 13.

(Parfenova, I. P.) *Парфенова И. П.* (1950). Лимфатическая сеть в пределах легочной дольки. Докл. АН СССР 72, 613

(Parfenova, I. P.) *Парфенова, И. П.* (1952). Лимфатическая система нормального легкого в анатоморентгенологическом отображении. Пробл. туберк. 1, 20

Parker, R. B. (1954). Meigs' syndrome. Lancet 2, 242.

Parsons, R. J. & Ph. McMaster (1938a). The effect of the pulse upon the formation and flow of lymph. J. Exp. Med. 68, 353

Parsons, R. J. & Ph. D. McMaster (1938b). Normal and pathological factors influencing the spread of vital dye in the connective tissue. J. Exper. Med. 68, 689.

Pasquale, E. L. di & A. A. Schiller (1951). Effect of hypoxaemia on edema formation *in perfused* isolated rat hind limb. Proc. Soc. Exp. Biol. Med. 78, 567.

Paschutin, W. W. (1872), Ueber die Absonderung der Lymphe im Arme des Hundes. Arb. Physiol Anst. Leipzig. 7, 197.

Patek, P. R. (1939), Morphology and lymphatics of mammalian heart. Am. J. Anat. 64, 203.

Pawlowsky, A. D. (1909), Das Schicksal einiger pathogener (hauptsächlich pyrogener) Mikroben bei ihrem Eindringen in den Tierorganismus von den Gelenken, der Pleura, dem Auge, der Mundhohle, dem Darmkanale und der Vagina aus. Ztschr. Hyg u Infectionskr. 62, 433.

Peabody, H. D., H. J. Moersch & J. E. Edwards (1951), Clinically indeterminate pulmonary fibrosis: a pathologic study. Proc. Staff. Mett. Mayo Clin. 26, 266.

Peabody, J. W., J. W. Peabody Jr., E. W Hayes & E. W. Hayes Jr. (1950), Idiopathic pulmonary fibrosis, its occurrence in identical twin sisters. Dis. Chest. 18, 330.

Pearse, A. G. E. (1954), Histochemistry. Churchill, London.

Pecquet, J. (1651), Experimenta nova anatomica, quibus incognitum chyli receptaculum et ab eo p...

Pekelharing, C. ...
sur le courant lympha...

Pensa, A. (1908), ...
chili et del ductus tho...
norm Roma. 14, 1.

Pepere, A. (1906), Su tre casi di rottura della vena coronaria del enora. Clin. mod Firenze. 12, 385.

Peremeschko (1867), Ein Beitrag zum Bau der Schilddruse. Z. wiss. Zool. 17, 286.

Perlmann, G. E, W. W. L. Glenn & D. Kaufman (1943), Changes in the electrophoretic pattern in lymph and serum in experimental burns. J. Clin. Investig. 22, 627.

Pessoa, V. C, K S Kim, & A. C. Ivy (1953), Fat absorption in absent of bile and pancreatic juice. Am. J Physiol. 174, 209.

Peters, J. P (1935), Body water. Ballière, Tindall & Co., London

Petersen, W. F. & S. A. Levinson (1923), The role of the endothelium in canine anaphylactic shock J Immunol. 8, 349

Petersen, W. F., R H. Jaffé, S. A. Levinson & T. P. Hughes (1923a), & Studies in endothelial permeability. III. The modification of the thoracic lymph following portal blockade. J. Immunol. 8, 361.

Petersen, W. F., R. H. Jaffé, S A. Levinson & T. P. Hughes (1923b), Studies in endothelial permeability. V. The effect of peptone on the permeability of the endothelium. J. Immunol 8, 377.

Petersen, W. F. & T. P. Hughes (1925), Effect of dextran- and levosuprarenin, pituitrin and pilocarpin on the mineral balance of the lymph. Journ. Pharmacol. 25, 137.

Petersen, W. F & T. P. Hughes (1925), Mineral metabolism of the lymph following injections of levo- and dextran suprarenin, pituitrin and pilocarpin. J. Biol Chem 66, 229.

Peterson, R. E. & J. D Mann (1952), Transport of radio-active iron in interstitial lymph. Am. J. Physiol 169, 763

Petrányi, Gy (1958), Hamman-Rich syndroma (Hamman-Rich syndrome). Orvosi Hetil. (Hungarian), 99, 1354.

Petroff, J. R. (1923), Über die Vitalfarbung der Gefäßwandungen. Beitr. path. Anat. u. allg. Path. 71, 115.

(Petrovski, V. V.) *Петровский, В В.* (1954), О роли лимфатических сосудов в кровообращении Физиол. журн СCC 40, 322

Pfaff, W. & W. Herold (1937), Grundlagen einer neuen Therapieforschung der Tuberkulose. Leipzig.

... der Anteil
74, 670.
Leber-

Pfuhl W. & W. Wirgand (1940), Die Lymphgefäße des großen Netzes beim Meerschweinchen und ihr Verhalten bei intraperitonealer Trypanblauinjektion. Klappenapparat und Mikrolymphherzen. Z. mikr.-anat. Forschg. 47, 117.
Phillips, R. A., V. P. Dole, P. B. Hamilton, K. Emerson Jr., R. M. Archibald & D. D. VanSlyke (1946), Effects of acute hemorrhagic and traumatic shock on renal functions of dogs. Am. J. Physiol. 145, 314.
Pic, A. W., B. J. Anson & H. W. Burnett (1944), Communications between lymphatic and venous system at renal level in man. Quart. Bull. Northw. Univ. Med. School. 18, 307.
Pigalew, I. (1928), Methoden zur Trennung des Subarachnoidalraumes bei Hunden im chronischen Experiment. Zeitschr. ges. exp. Med. 61, 1.
Podbelsky (1892), Über das Vorkommen des Kolloids in den Lymphgefäßen der strumös erkrankten menschlichen Schilddrüse. Prager Med. Wochenschr 197, 211.
Poirier, P. & B. Cunéo (1902), Les lymphatiques. In Poirier et Charpy, Traité d'anatomie humaine, Paris
Pólya, A. E. & D. Navratil (1903a), Untersuchungen über die Lymphbahnen der Wangenschleimhaut. Dtsch. Ztschr. Chir. 66, 122
Pólya, E. & D. Navratil (1903b), Untersuchungen über die Lymphbahnen des Wurmfortsatzes und des Magens. Deutsch. Zeitschr. Chir. 66, 421.
Ponfick, E. (1910), Über Hydronephrose. Zieglers Beitr. 49, 127.
Popoff, W. N. (1895), Zur Frage der Lymphbildung. Centralbl. Physiol. 9, 52.
Poppe, J. K. (1941), Reproduction of ulcerative colitis in dogs. Arch. Surgery. 43, 551.
Popper, H. (1937), Über experimentelle Hepatitis. Virchows Arch. 298, 574.
Popper, H. (1951), Liver Injury. 10th Conf. J. Macy Found. New York.
Popper, H. (1952), Interrelation between hepatic lesions and lesions of the biliary tract. J. Intern. Coll. Surgeons. 18, 743
Popper, H. (1953), Beziehungen zwischen Leberfunktion und Leberstruktur. Wien. Klin. Wschr. 65, 722
Popper, H. L. & H. Necheles (1940), Pathways of enzymes into the blood in acute damage of the pancreas. Proc. Soc. Exp. Biol. Med. 43, 220.
(Pozharski, P., N. Toltchskaya & A. Shilova), Пожарский, П. Н. Толчская и А. Шилова (1950) Патологическая анатомия силикоза. Арх. патологии 2, 13
Potter, B. P. & I. E. Gerber (1948), Acute diffuse interstitial fibrosis of the lungs; report of case. Arch. Int. Med. 82, 113.
Poynter, C. W. M. (1928), Concerning the great omentum. Med. Clin. N. Amer. 12, 499.
Pratt, F., M. Reid (1930), The lymph heart as a facultative effector. J. Physiol. 93, 681.
Pratt, F., M. Reid (1932), Homolateral synchronism of lymphatic hearts. Proc. Soc. Exp. Biol. Med. 29, 1019
Pratt, G. H. & L. K. Ferguson (1947), Cicatrizing enterocolitis. Am. J. Surgery. 73, 28
Prentice, T. C., W. Siri & E. E. Joiner (1952), Quantitative studies of ascitic fluid circulation with tritium-labelled water. Am. J. Med. 13, 668.
Priestley, J. (1878), An account of the anatomy and physiology of batrachian lymph hearts. J. Physiol. 1, 19
Poynter, C. W. M. (1928), Concerning the great omentum. Med. Clin. N. Amer.
Primak, F. (1910), Das Verhalten des Herzens im Alter und bei Herzkranken. Starost. Kiev. 97.
(Prives, M. G.) Привес, М. Г. (1948), Рентгенография лимфатической системы. Ленинград
(Prives, M. G.) Привес, М. Г. (1955) Коллатеральное лимфообращение при нарушении нервной системы. Арх. анат. гистол. и эмбриол. 4/74 82.
Probstein, J. G. & G. E. Gruenfeld (1936), Acute regional ileitis. Am. J. Surg. 103, 273.
Pullinger, B. D. & H. W. Florey (1935), Some observations on the structure and functions of lymphatics; their behaviour in local oedema. Brit. J. Exp. Path. 16, 49.

Putnam, T. J. (1923), The living peritoneum as a dyalizing membrane Am J. Physiol. 63, 548.

Quincke, H. (1875), Über fetthaltige Transsudate. Dtsch. Arch. klin. Med 16, 121.

Quincke, H. (1872), Zur Pathologie der cerebrospinalen Flussigkeit. Arch Anat. Physiol u. wiss. Med 153.

Quinn, R W. (1948), Antihyaluronidase studies of serums from patients with rheumatical fever, streptococcal infections and miscellaneous non-streptococcal diseases. J. Clin. Investig. 27, 471.

(Rabinovitch, E. A.) *Рабинович, Е. А* (1935), Возрастные особенности лимфообращения висцеральной плевры и легкого. Пробл туберк 9, 40

Radetzky (1914), Zur Frage u d. Zusammenhang des Subarachnoidalraums d. zer. u spin Marks mit dem Lymphsystem d. menschl. Korpers Russkij Wratsch.

Radziejewski, S (1868), Experimentelle Beitrage zur Fettresorption. Virchows Arch 43, 268.

Ragan, C. (1953), Connective tissues Transact 4th Conference J. Macy Jr Found. New York.

Rajka, Ö. (1926), Über das Messen des Capillardruckes an der menschlichen Haut mit dem Torok—Rajka—Wessely'schen Capillartonometer Z ges. exp. Med. 48, 570.

Ramsdell, S. G (1928), The use of trypan blue to demonstrate the immediate skin reaction in rabbits and guinea pigs J. Immunol. 15, 305

Randerath, E (1937), Die Entwicklung der Lehre von den Nephrosen in der [illegible] und path Anat. 32, 91

[illegible] der Paraproteinosen Verh. deutsch [illegible] Stuttgart.

[illegible] tercapillaren (diabetischen) Glomerulosklerose Virchows Arch. 32[illegible], 4[illegible].

Ranke, J (1865), Der Tetanus. Engelman, Leipzig.

Ranson, S W, L R. Faubion & C J. Ross (1923), Vasodilator mechanism IV. The intraarterial injection of histamine. Am J. Physiol. 64, 311.

Ranvier, L. (1873), Du système lymphatique Leçons recueillies par le dr. Weber. Progrès Med. I, 25, 51, 73, 99, 145, 181, 206

Ranvier, L (1895), Développement des vaisseaux lymphatiques C. R. Acad Sci 121, 1105.

Ranvier, L (1896a), Aberration et régression des lymphatiques en voie de développement. C. R. Acad Sci. 122, 578

[illegible] la mor- [illegible] t et le lapin C. R. Acad Sci. 123, 923

Ranvier, L (1896d), La théorie de la confluence des lymphatiques et le développement des ganglions lymphatiques C R Acad Sci. 123, 1038.

Rappaport, H. & F. H. Burgoyne (1948), Histologic lesions encountered in segmental enteritis. Am. J. Path 24, 725

Rather, L J (1952), Filtration, resorption and excretion of protein by the kidney. Medicine 31, 357.

(Ravitch-Shtcherbo, V. A) *Равич-Щербо, В. А* (1940), О физиологическом [illegible] Сб »Ретроградный ги- [illegible]

[illegible] urally related diazo dyes [illegible]

[illegible] ihre Beziehungen zum [illegible] -hows Arch 26, 172.

Recklinghausen, F. [illegible]

Recklinghausen, F. (1869), »Lymphgefaßsystem«. In: Strickers Handb d. Gewebenlehre

Recklinghausen, F (1885), Über die venose Embolie und den retrograden Transport in den Venen und in den Lymphgefaßen. Virchows Arch. 100, 503.

Recklinghausen, H. (1906). Unblutige Blutdruckmessung. Arch. exp. Path. u. Pharmakol. 1, 375.

Reddingius (1912). Über gegenseitigen Übergang von Fettläppchen in Lymphdrüsenknoten. Centralbl. allg. Pathol. 23, 441.

Regéczy, E. N. (1885). Die Ursache der Stabilität des Blutdruckes. Pflugers Arch. 37, 73.

Rehn, L. (1913). Zur experimentellen Pathologie des Herzbeutels. Arch. klin. Chir. 102, 1.

Reichert, F. L. (1926). The regeneration of the lymphatics. Arch. Surgery 13, 871.

Reichert, F. L. & M. E. Mathes (1936). Experimental lymphedema of the intestinal tract and its relation to regional cicatrizing enteritis. Ann. Surgery 104, 601.

Reid M.: Automatism of anuran lymph hearts as obtained by transplantation. Proc. Soc. Exp. Biol. Med. 30, 667, 1933.

Reid M.: Automaticity of transplanted anuran lymph hearts. J. Exp. Zool. 76, 47, 1937.

Reinhardt, K. (1953). A propos d'un cas de lymphangiectasie pulmonaire. Radiologia Clinica 22, 162.

Reinhardt, W. O. & C. H. Li (1945). Depression of the lymphocyte content of the thoracic duct lymph by adrenocorticotrophic hormone. Science 101, 360.

Reinhardt W. O. & Li C. H. (1945). Cell count, rate of flow and protein content of cervical lymph in the rat. Proc. Soc. Exp. Biol. Med. 58, 321.

Reinhardt, W. O., M. C. Fisher & I. L. Chaikoff (1944). The circulation of plasma phospholipids, their transport to thoracic duct lymph. J. Biol. Chem. 152, 79.

(Reingold, M. L.) *Рейнгольд, М. Л.* (1944). К вопросу о ретроградном медиастинокардиальном токе лимфы. Сб. «Ретроградный лимфоток эндоторакальной области». Воронеж.

Reinmuller J. (1935). *Über die Automatie der coccygealen Lymphherzen bei Rana fusca und Rana esculenta.* Pflügers Arch. 236, 275.

*Remak, R.* (1850). Ueber blutleere Gefäße (Lymphgefäße) im Schwanze der Froschlarve. Arch. Anat. Physiol. u. wiss. Med. 102.

Remington, J. W., N. C. Wheeler, G. H. Boyd Jr. & H. M. Caddell (1948). Protective action of dibenamine after hemorrhage and after muscle trauma. *Proc. Soc. Exp. Biol. Med.* 69, 150.

Remington, J. W., W. F. *Hamilton, H. M. Caddell, G. H. Boyd Jr., N. C.* Wheeler & R. W. Pickering (1950). Vasoconstriction as a precipitating factor in *traumatic shock in the dog. Am. J.* Physiol. 161, 125.

Renaut, J. (1889/90). Traité d'histologie pratique. Lecrosnier et Babé, Paris.

Renkin, E. M. (1953). Capillary and cellular permeability to some compounds related to antipyrine. Am. J. Physiol. 173, 125.

Renkin, E. M. (1954). Permeability and structure of inert porous membranes. Federat. Proc. 13, 116.

Renkin, E. M. & B. D. Zaun (1955). Effect of adrenal hormones on capillary permeability *in perfused rat* tissues. Am. J. Physiol. 180, 498.

Renvers (1890). Über Ascites chylosus. Berliner Med. Wschr. 27, 320.

Rényi-Vámos, F. (1950a). Az interstitium és a nyirokerek jelentősége az ascendáló pyelonephritisben (Significance of interstitial space and lymphatics in the ascending pyelonephritis). Kísérl. Orvostud. (Hungarian), 2, 152.

Rényi-Vámos, *F. (1950b).* A vese nyirokereinek *anatómiája (Anatomy of* renal lymphatics) Kísérl. Orvostud. (Hungarian), 2, 331.

*Rényi-Vámos,* F. (1950c). A nyirokerek viselkedése emberi vese fertőzéses megbetegedéseiben (Behaviour of lymphatics in the infectious diseases of the human kidney) Kísérl. Orvostud. (Hungarian), 2, 334.

Rényi-Vámos, *F. (1950d). Kísérleti* adatok a vesetok fejlődéséhez (Experimental data concerning development of the kidney capsule). Kísérl. Orvostud. (Hungarian), 2, 425.

Rényi-Vámos, F. (1951), Über die »lymphogene« Aszension der Pyelonephritis Acta Medica Hung. 3, 7.

Rényı-Vámos, F. (1952), A vese nyırokkeringésének insufficientiája (Insufficiency of renal lymph circulation). MTA V. Oszt. kozl. (Hungarian), 3, 493.

Rényı-Vámos, F. (1954), Újabb vizsgálatok egyes szervek nyırokrendszere terén (New investigations of the lymphatic system of certain organs) Budapest. Dissertation in Hungarian.

Rényı-Vámos, F. (1955), Über einige Probleme der Lymphforschung Acta Morph Hung. 6, 71.

Rényı-Vámos, F. (1955), A here és a mellékhere nyirokrendszere (Lymphatic system of the testis and epididymis). MTA V. Oszt. kozl. (Hungarian), 6, 83

Rényı- … Dunndarms und seine Rolle im F …

Rényı- … vese és az uregrendszer nyırokerein… után (Demonstration and signific… pelvic cavity after the obstruction of the renal pelvis) Kísérl. Orvostud (Hungarian), 1, 198

Rényi-Vámos, F , F Balogh & Z Szendrőy (1950a), A vese uregrendszerének felszívódási viszonyai (Conditions of absorption in the cavities of the kidney) Kísérl. Orvostud. (Hungarian), 2, 21

Rényı-Vámos, F., F. Balogh & Z Szendrőy (1950b), A pyelonelzáródás néhány problémája (Some problems regarding the occlusion of the pelvis). Orvosi Hetil (Hungarian), 91, 646.

Rényı- … Gallenblase und seine … ), 295

Rényı- … Duodenitis bei experim…

… ói … is … l- … n … n … g-

11, 87.

Rényi-Vámos F. & Gy. Szinay (1958), Das Lymphgefaßsystem des Coecums. Acta Anat 34, 124.

… logic … ment … ment … 24, 127.

Ricca, R. A , K. Fink, L. T. Steadman & S. L. Warren (1945b), Distribution of body fluids of dogs in traumatic shock. J. Clin. Invest. 24, 140.

Rich, A. R. (1921), Condition of the capillaries in histamine shock. J. Exp. Med. 33, 287.

Rich, A R. (1951), The pathogenesis of tuberculosis. 2. ed Springfield

Richter, C. P. (1952), The physiology and cytology of pulmonary edema and pleural effusion produced in rats by alfa-naphthyl-thiourea (ANTU). J. Thor. Surg. 23, 66.

Richter, H. (1907), Eine Untersuchung über den histologischen Bau des Ductus thoracicus des Pferdes. Berl. tierärztl. Wschr. 213.

Ricker, O. (1924), Die Pathologie als Naturwissenschaft. Springer, Berlin.

Riley, J. F. (1954), The riddle of the mast cells Lancet 1, 841.

Rindowski, D. F. (1896), Die Lymphgefäße der Niere. Zentralbl. Med. Wissenschaft. 7, 145.

(Rindovski, D. F.) *Риндовский, Д. Ф* (1871), Лимфатические сосуды почек. Труды III. съезда русских естествоиспытателей. Отд. научн. мед 57.

*Ritter, C. (1900), Für Neubildung der Lymphdrüsen, Entgegnung auf die* Arbeit von Bartels über Neubildung von Lymphdrüsen in der Cubitalgegend. Arch. Anat. und. Physiol. Anat. Abt. 300

Ritter, C. (1905), Die Neubildung von Lymphdrüsen beim Mammacarcinom Deutsche Ztschr. Chir. 79, 260.

Robertson, W. B., M. W Ropes & W Bauer (1940), Mucinaze: a bacterial enzyme which hydrolizes synovial fluid mucin and other mucins. J Biol Chem. 133, 261.

Robertson, W. V., M W. Ropes & W. Bauer (1914), Degradation of mucins

Rogowicz, N. (1885), Beiträge zur Kenntnis der Lymphbildung. Pflügers Arch. 36, 252.

Rodrigues, A , R. Carvalho & S. Pereira (1933), Le canal thoracique et ses voies collatérales C. R. Ass d Anat 1.

Rombányi, Gy. (1949), Über die submikroskopische Struktur des Amyloids Schweiz. Ztschr. Path Bact 12, 253.

Romualdi, G. & M. Monaci (1947a), Le modificazioni della funzione dei reni in corso di linfostasi in animali normali e nell' ipertrofia vicariante Arch «de Vecchi» 9, 973.

Romualdi, G. & M Monaci (1947b), La nefrosi consecutiva a linfostasi sperimentale e la nefrosi da tossici esogeni nel rene in linfostasi Arch. «de Vecchi» 9, 987.

Róna, Gy. (1953), A glomerulosklerosis (The glomerulosclerose). Budapest. Dissertation.

Rony, H. R , B Mortimer & A. C. Ivy (1932), Fat transport through the lymph system in fasting and phlorizin poisoning J Biol Chem. 96, 737.

Rosenfeld, L., J L Langohr, C. R Owen & O Cope (1949), Circulation of the blood and lymph in frostbite and influence of therapeutic cold and warmth Arch. Surgery 59, 1045

of th

Path. Ges. 23 Tagung 89

ith Anat.

dowschen Virchows

Rossle, R. (1944), Über die serose Entzundung der Organe Virchows Arch. 311, 252

(Rotenberg, A. L ) *Ротенберг, А Л* (1949), Слияние отводящих лимфатических сосудов печени и желудка Труды Ленингр сан.-гиг. мед ин-та 3, 160.

Rényi-Vámos, F. (1951), Über die »lymphogene« Aszension der Pyelonephritis. Acta Medica Hung. 3, 7.

Rényi-Vámos, F (1952), A vese nyirokkeringésének insufficientiája (Insufficiency of renal lymph circulation) MTA V. Oszt. közl (Hungarian), 3, 493.

Rényi-Vámos, F (1954), Újabb vizsgálatok egyes szervek nyirokrendszere terén (New investigations of the lymphatic system of certain organs). Budapest. Dissertation in Hungarian.

Rényi-Vámos, F. (1955), Über einige Probleme der Lymphforschung Acta Morph Hung. 6, 71

Rényi-Vámos, F. (1955), A here és a mellékhere nyirokrendszere (Lymphatic system of the testis and epididymis) MTA V. Oszt. kozl. (Hungarian), 6, 83

Rényi-Vámos, F. (1956), Das Lymphgefäßsystem des Dunndarms und seine Rolle im Fetttransport. Acta. Med. Hung. 9, 153.

Rényi-Vámos, F., F. Balogh & Z Szendrõy (1949), A vese és az üregrendszer nyirokereinek kimutatása és jelentõsége pyelon elzáródás után (Demonstration and significance of the lymphatics of the kidney and the pelvic cavity after the obstruction of the renal pelvis). Kísérl. Orvostud. (Hungarian), 1, 198

Rényi-Vámos, F., F Balogh & Z. Szendrõy (1950a), A vese üregrendszerének felszívódási viszonyai (Conditions of absorption in the cavities of the kidney) Kísérl Orvostud (Hungarian), 2, 21

Rényi-Vámos, F., F. Balogh & Z Szendrõy (1950b), A pyelonelzáródás néhány problémája (Some problems regarding the occlusion of the pelvis). Orvosi Hetil (Hungarian), 91, 646

[illegible] Gallenblase und [illegible], 295.

[illegible] Duodenitis bei [illegible]

Rényi-Vámos, F., P. Magasi & E Mátyus (1954), Der Histamingehalt der [illegible]

[illegible] 11, 87.

Rényi-Vámos F. & Gy. Szinay (1958), Das Lymphgefaßsystem des Coecums Acta Anat. 34, 124.

Rhinelander, R W., J. L. Langohr & O. Cope (1949), Explorations into physiologic basis for therapeutic use of restritive bandages in thermal trauma, experimental study. Arch. Surgery. 59, 1056.

Ricca, R A., K Fink, L. L. Katzin & S. L. Warren (1945a), Effect of environmental temperature on experimental traumatic shock in dogs. J. Clin. Invest. 24, 127.

Ricca, R. A., K. Fink, L. T. Steadman & S. L. Warren (1945b), Distribution of body fluids of dogs in traumatic shock. J. Clin Invest. 24, 140.

Rich, A. R. (1921), Condition of the capillaries in histamine shock. J. Exp Med. 33, 287.

Rich, A. R. (1951), The pathogenesis of tuberculosis 2. ed. Springfield.

Rusznyák, I., M. Földi & Gy. Szabó (1955), Physiologische und pathologische Bedeutung des Lymphkreislaufes. Schweiz. med. Wschr 85, 1037

Rusznyák, I., M. Földi & Gy. Szabó, Sur la circulation lymphatique de quelques glandes endocrines. Presse Médicale.

Rusznyák, I., I. Karády & A. Hámori (1942), A traumas shock keletkezése (Origin of traumatic shocks) Magyar Orv. Arch (Hungarian) 43 431

Rusznyák, I. & A. Szentgyörgyi (1936), Vitamin P Flavonols as vitamins Nature 138, 27.

Ruyschius, F. (1712), Dilucidatio valvularum in vasis lymphaticis et lacteis In Mangeti bibl. anat. II. 712.

Ryle, J. A. (1924), Fatty stools from obstruction of the lacteals with a note on the coeliac affection. Guy's Hosp. Rep 74, 1.

Sabin, F. R. (1902), On the origin of the lymphatic system from the veins and the development of lymph hearts and thoracic duct in the pig Am J Anat 1, 367.

Sabin, F. R. (1908), Further evidence on the origin of the lymphatic endothelium from the endothelium of the blood vascular system Anat Rec 2, 46

Sager, W. W. & A. C. Nickel (1929), Localization of bacteria in tissues of lowered resistance. Arch. Surgery 19, 1086.

Sahli, H. (1885), Zur Pathologie und Therapie des Lungenodems Arch exp Path. u Pharmakol. 19, 433.

[illegible]

Sala, L. (1900), Sur le développement des coeurs lymphatiques et des conduits thoraciques dans l'embryon de poulet. Arch. ital Biol. 34, 453.

Saltzmann, J. (1711), Dissertatio anat. exhibens Encheirisin Novam qua ductus thoracicus una cum receptaculo chyli in quovis subiecto humano demonstrari potest. Arg.

Samuel, S. (1890), Über anaemische, hyperaemische und neurotische Entzündungen. Virchows Arch 121, 396.

Samuelsen, G. S., G. E. Griffin, E Munwyler & E. Seifter (1948), Absorp- [illegible] 277.

[illegible] méd 942.

[illegible] gie des vaisseaux

[illegible] ve & E. Lecroisnier, Paris

Sappey (1888), Traité d'anatomie descriptive 4. éd Masson, Paris

Sarnoff, S. J. & E Berglund (1952a), Neurohemodynamics of pulmonary edema. Effect of systemic vasoconstriction and subsequent vasodilation of low and pressure in systemic and pulmonary vascular beds. Am. J. Physiol 170, 588

Sarnoff, S. J. & E. Berglund (1952b), Pressure volume characteristics and stress relaxation in the pulmonary vascular bed of the dog Am J Physiol 171, 238

Sarnoff, S J, W. T. Goodale & L. C. Sarnoff (1952), Graded reduction of arterial pressure in man by means of a thiophanium derivate. (Ro 2—2222), Preliminary observations on its effect in acute pulmonary edema. Circulation 6, 63

Sarnoff, S J. & L. C. Sarnoff (1952), Neurohemodynamics of pulmonary edema The role of sympathic pathways in the elevation of pulmonary and systemic vascular pressure following the intracysternal injection of fibrin. Circulation 6, 51.

Saslow, G. (1938), The relation between the oxygenization of fluids and the occurrence of edema in the perfused frog web. Am. J. Physiol 124, 360

Saunders, W (1809), A treatise on the structure, economy and diseases of the liver, together with an inquiry into the properties and component parts of the bile and biliary concretions With an inquiry into the properties and component parts of bile and biliary calculi. 4. ed. London

Saxser, F (1896), Über die Entwicklung und den Bau der Lymphdrusen und die Entstehung der roten und weißen Blutkörperchen Anat. Hefte 6, 347.

(Rotenberg, A. L.) *Ротенберг, А. Л.* (1952), Внутриорганная лимфатическая система легкого Труды Ленингр сан.-гиг мед инта **17**, 14.

Röth, W. (1899), *Ueber die Permeabilität der Capillarwand und deren Bedeutung* fur den Austausch zwischen Blut und Gewebsflussigkeit Arch. Anat u. Physiol, Physiol Abt. 416.

Rotman (1897), Über fetthaltige Ergusse in den großen serosen Hohlen. Zeitschr. klin. Med. **31**, 416.

the t

their

of st

abili

Rous, P. & F. Smith (1938), The gradient of vascular permeability. *III.* The gradient along the capillaries and venules of the frog skin. *J* Exp. Med. **53**. *219*.

Rouvière, H. (1929), Sur les lymphatiques des ganglions sympathiques cervicaux (note préliminaire) Ann. d'Anat pathol. et d'Anat norm. méd. chir. **6**, 222.

Rouvière, H. (1932), Anatomie des lymphatiques de l'homme. Masson & Cie., Paris

Rouvière, H. & G Valette (1933), Rôle des ganglions lymphatiques dans la circulation de la lymphe. Bull. Acad. Méd. **110**, 189.

Rouvière, H. & G. Valette (1935), La pression lymphatique. C. R. Soc Biol *118*, *1398*.

Rouvière, H & G Valette (1937), Physiologie du système lymphatique. Masson & Cie., Paris

Roy, C. & G. Brown (1879), The blood pressure and its variations in the arterioles, capillaries and smaller veins J Physiol. 2, 323.

Rubin, E. H, B S. Kahn & D Pecker (1952), Diffuse interstitial fibrosis of the lungs Ann. Int Med **36**, 827.

Rudbeck, O (1653), Nova exercitatio anatomica exhibens Ductus Hepaticos Aquosos et Vasa Glandularum Serosa, nunc primum in venta, aeneisque figuris delineata Arosiae

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Kylin: Die Eiweißkor

(Rusznyák, I.) *Р*

и патологии лимфоо(

Rusznyák, I. (19

reo-

lazione linfatica. Minerva Medica **45**, *I*. *41*.

Rusznyák, I., J. Baráth & I Kürthy (1924), Untersuchungen über die klinische Bedeutung der Eiweißfraktionen des Blutplasmas Z. klin. Med. **98**, 337.

Rusznyák, I. & S. Benkő (1941), Vitamin-e a citrin? (Is citrin a vitamin?) Lecture held in Hungarian.

Rusznyák, I, M. Földi & Gy. Szabó (1949), Lymphangiospasmus (Lymphangiospasm). Kísérl. Orvostud. (Hungarian), **1**, 44.

Rusznyák, I., M. Földi & Gy. Szabó (1950), Lymphangiospasm. Acta Med. Scand. **137**, 37.

Servelle, M. (1952), Pathologie vasculaire médicale et chirurgicale Masson & Co., Paris.

Servelle, M., M. Albeaux-Fernet, S. Laborde, J. Chabot & J. Rouguelle (1957), Lésions des vaisseaux lymphatiques dans les malformations congénitales des veines profondes. Presse Méd. 65, 531.

Shackleford, R. T. & A. M. Fisher (1938), Traumatic chylothorax. South. M. J. 31, 766.

Shafiroff, B. G., P. Doubilet, A. L. Preiss & Co Tui (1943), Effect of thoracic duct drainage and hemorrhage on blood and lymph Surg., Gynec. & Obst 76, 547.

Shafiroff, B. G. P., H. Doubilet & W F Rouggiero (1939), Bilirubin resorption in obstructive jaundice Proc. Soc. Exp. Biol. Med 42, 203.

Shafiroff, B. G. P. & J. R. Bierman (1940), Resorption of radio-opaque substances from experimentally obstructed gall bladder Am. J. Physiol. 129, 703.

Shafiroff, B. G. P., H Doubilet, W. F. Rouggiero, A P Preiss & Co Tui (1942), The effect of lymphatic block on bile resorption in obstructive jaundice Am. J. Physiol. 137, 97

Sharpey-Schafer E. (1929), Relationship between the liver cells and the blood. J. Physiol 67. XL.

Shinghu, S. (1908), Beiträge zur Physiologie des künstlichen Pneumothorax und seiner Wirkung auf die Lungentuberculose. Beitr klin tuberc. 9, 1

Shipley, P G. & R. S. Cunningham (1916), Studies on absorption from serous cavities. I The omentum as a factor in absorption from the peritoneal cavity Am J Physiol 40, 75.

Sholl, A. T (1939), Mineral metabolism, Reinhold, New York

Shore, L. E. (1890), On the fate of peptone in the lymphatic system. J Physiol. 11, 528.

Shuman, C R. & A. J Finestone (1950), Inhibition of hyaluronidase in vivo by adrenal cortical activation. Proc. Soc Exp Biol. Med. 23, 248

Siegmund, H. (1942), Zur pathologischen Anatomie der Hepatitis epidemica Münch med. Wschr 89, 463

(Sikorski, N.) Сикорский, Н. (1872), О лимфатических сосудах легких Киев

Silvester, C. F. (1912), On the presence of permanent communications between the lymphatic and the venous system at the level of the renal veins in adult South-American monkeys Am. J Anat. 12, 447

[illegible]
to it- [illegible]

in the [illegible]

Simer, P. H. (193[illegible]), [illegible]

Simer, P H (1947), The drainage of particulate matter from the peritoneal cavity by the lymphatics Anat. Records 88, 175.

Simer, P. H. (1948), The passage of particulate matter from the peritoneal cavity into the lymph vessels of the diaphragm. Anat. Records 101, 333

Simon, N. & L Narins (1949), The effect of hyaluronidase on the absorption of a subcutaneously deposited radio-opaque substance. Am J. Roentgenol 61, 91

Simmonds, W J. (1953), The absorption of labelled erythrocytes from the [illegible] J. Exper. Biol. & Med Sci 31, 77

[illegible]

failure to produce [illegible]

Sisganov, A [illegible]
beim Menschen [illegible]

(Sisganov, A [illegible]
и почечных оболочек человека. Алма Ата.

Schade, H. & F. Claussen (1924), Der onkotische Druck des Blutplasmas und die Entstehung der renal bedingten Ödeme. Z. klin. Med. 100, 363.

Schade, H. & H. Menschel (1923), Über die Gesetze der Gewebsquellung und ihre Bedeutung fur klinische Fragen. (Wasseraustausch im Gewebe, Lymphbildung und Oedementstehung.) Ztschr. klin. Med 96, 279.

(Shaklein, I. A.) *Шаклейн, И А.* (1950), Значение лимфатической системы легких при лечебном пневмоторатсе. Пробл туберк 1, 31.

Schechter, A. J. (1931), Electrolyte and volume changes in fluids injected into the peritoneal cavity. Yale J. Biol. 4, 167.

Schechter, A. J., M. K. Cary, A. L Carpentieri & D. C. Danowski (1933), Changes in composition of fluids injected into peritoneal cavity Am J. Dis Child. 46, 1015.

Schepers, G. W H. (1945), The pathology of regional ileitis Am J. Digest Dis. 12, 97

Scherf, D. & L. J Boyd (1948), Cardiovascular diseases. W. Heinemann, London.

Schmidt, C. (1850), Charakteristik der epidemischen Cholera gegenuber verwandten Transsudationsanomalien. A. Reyher, Leipzig u. Mitau.

Schmidt, C. F. & M. Hayman Jr (1929), A note upon lymph formation in the dogs kidney and the effect of certain diuretics upon it Am. J. Physiol 91, 157.

Schoenberger, J. A., R. M Kark, G Kroll & E. Eckert (1953), Endothelial permeability in man studied with albumin $I^{131}$ injected intravenously or intraperitoneally. J Clin. Investig 32, 601.

Schoenberger, J. A., G. Kroll, A. Sakamoto & R. M Kark (1952), Investigation of the permeability factor in ascites and edema using albumin tagged with $I^{131}$. Gastroenterology 22, 607

Schubert, R. (1949), Serumsanieren mit künstlichen Kolloiden. Dtsch. Med Wschr 74, 1489.

Schubert, R (1951), Die Anwendung von Periston N zur Serum- und Zellwäsche und ihre klinische Bedeutung. Dtsch Med. Wschr 76, 1487.

Schulz, Fr N. (1925), Bildung der Lymphe. In. Handb. der Biochemie des Menschen und der Tiere, [illegible]

Schulze, W (1925), [illegible] und post-capillären Venen lymphatischer Org [illegible] 76, 421.

Schulze, W. & H May (1931), Über die Einwirkung lungeneinengender Operationen auf Blut- und Lymphkreislauf der Kaninchenlunge Deutsch. Ztschr. Chirurg 230, 86.

Schwalbe, G. (1869), Der Arachnoidalraum, ein Lymphraum und sein Zusammenhang [illegible] nschaft. 7, 465.

Schwa[illegible] ender und tätig gewesener [illegible] hem. Z. 37, 34.

Scott, [illegible] influencing the interchange of fluid between blood and tissue spaces II. Muscular activity. Am J Physiol 44, 313.

Seifried, O. (1924), Lymphknoten und Tuberkulose bei Vogeln, insbesondere bei Huhnern. Arch Tierheilk 51, 425.

Seiffer, J. & D. H. Baeder (1954), Partially depolymerized hyaluronic acid (PDHA) as a spreading agent Proc. Soc. Exp. Biol. Med 85, 160

Selkurt, E. E. (1946), Renal blood flow and renal clearance during hemorrhagic shock. Am. J Physiol. 145, 699

Selye, H (1946), General adaptation syndrome and diseases of adaptation J. Clin Endocrinol. 6, 117.

Selye, H. (1950), The physiology and pathology of exposure to stress. Thérien, Montreal.

Senator, H (1895), Ascites chylosus and Chylothorax duplex. Carcinom des Ductus thoracicus Charité-Annalen. 20, 263.

Serr, H. (1924), Blutbeschaffenheit und Glaukom. Messungen des osmotischen Druckes der Bluteiweißkorper bei Glaukomkranken, sowie experimentelle Untersuchungen uber die Rolle des osmotischen Druckes der Bluteiweißkorper fur den normalen und pathologischen Flussigkeitswechsel. Arch. Ophth. 114, 393

Servelle, M. (1952), Pathologie vasculaire médicale et chirurgicale. Masson & Co, Paris.
Servelle, M, M Albeaux-Fernet, S. Laborde, J. Chabot & J. Rouguelle (1957), Lésions des vaisseaux lymphatiques dans les malformations congénitales des veines profondes Presse Méd. 65, 531.
Shackleford, R. T. & A. M. Fisher (1938), Traumatic chylothorax. South. M. J. 31, 766.
Shafiroff, B. G., P. Doubilet, A L. Preiss & Co Tui (1943), Effect of thoracic duct drainage and hemorrhage on blood and lymph. Surg, Gynec. & Obst. 76, 547.
Shafiroff, B. G. P., H. Doubilet & W. F. Rouggiero (1939), Bilirubin resorption in obstructive jaundice. Proc. Soc. Exp. Biol. Med 42, 203.
Shafiroff, B. G. P. & J. R. Bierman (1940), Resorption of radio-opaque substances from experimentally obstructed gall bladder. Am. J. Physiol. 129, 703
Shafiroff, B. G P., H. Doubilet, W. F. Rouggiero, A P Preiss & Co Tui (1942), The effect of lymphatic block on bile resorption in obstructive jaundice. Am J. Physiol. 137, 97.
Sharpey-Schafer E. (1929), Relationship between the liver cells and the blood. J Physiol 67. XL.
Shinghu, S. (1908), Beiträge zur Physiologie des kunstlichen Pneumothorax und seiner Wirkung auf die Lungentuberculose. Beitr klin tuberc. 9, 1.
Shipley, P. G & R. S Cunningham (1916), Studies on absorption from serous cavities. I. The omentum as a factor in absorption from the peritoneal cavity. Am. J Physiol 40, 75.
Sholl, A T. (1939), *Mineral metabolism*, Reinhold, New York.
Shore, L. E. (1890), On the fate of peptone in the lymphatic system J Physiol. 11, 528

(Sikorski, N) *Сикорский, Н* (1872), О лимфатических сосудах легких Киев

Simer, P H (1935), Omental lymphatics in man Anat Record 63, 253.
Simer, P H (1947), The drainage of particulate matter from the peritoneal cavity by the lymphatics Anat Records 88, 175
Simer, P H (1948), The passage of particulate matter from the peritoneal cavity into the lymph vessels of the diaphragm. Anat. Records 101, 333
Simon, N & L Narins (1949), The effect of hyaluronidase on the absorption of a subcutaneously deposited radio-opaque substance. Am J Roentgenol 61, 91
Simmonds, W J (1953), The absorption of labelled erythrocytes from the subarachnoid space in rabbits. Austr J Exper Biol & Med. Sci 31, 77.
Simmonds, J P & W W. Brandes (1927), Effect of mechanical obstruction of hepatic veins upon outflow of lymph from the thoracic duct J. Immunol 13, 11.
Sinaiko, E S & H Necheles (1946), Experiments in ulcerative enteritis; failure to produce it by mesenteric lymphatic obstruction. Surgery 20, 395.
Sisganov, A N (1930), Über das Lymphsystem der Nieren und Nierenhullen beim Menschen Ztschr. Anat Entwicklungsg. 91, 770.
(Sisganov, A N) *Сызганов, А Н* (1940), О лимфатической системе почек и почечных оболочек человека. Алма Ата

(Skvortsov, Y Y.) *Скворцов, Й. Й.* (1874), Материалы для анатоми и хистологии оболочек сердца. Санкт-Петербург.

Smetana, H. (1947), The permeability of the renal glomeruly of several mammalian species to labelled protein. Am J. Path. 23, 255.

Smetana, H. & F. R. Johnson (1942), The origin of colloid and lipoid ropleti in the epithelial cells of the renal tubules Am. J. Path. 18, 1029.

*Smith, A. L. (1941)*, Regional enteritis (terminal ileitis), review of 13 cases Brooklyn Hosp R. 3, 6.

Smith, H. W. (1951), The kidney. Structure and function in health and diseas Oxford. Univ Press, New York.

Smith, F & M. Dick (1932), The influence of the plasma colloids on the gradie of capillary permeability. J. Exp. Med. 56, 371.

Smith, M. I. (1927), Studies on experimental shock with especial reference to it treatment. J. Pharmacol. & Exper. Therap 32, 465

Smith, R. H. & T. J. Moran (1954), Experimental aspiration pneumonia III Pneumonia produced by intratracheal injection of carbohydrate solutions A M. A Arch. Path. 57, 194

Snook, T (1946), Deep lymphatics of the spleen. Anat Rec. 94, 43

Sollman, T. & J. D Pilcher (1916), Endermic reactions. J. Pharmacol. & Exper. Therap 9, 309

Sotnitschewsky (1879), Über Stauungs-Ödem. Virchows Arch 77, 85.

Speranski, A D. (1950), Grundlagen der Theorie der Medizin W. Saenger Berlin

Sperry, W. M, R. W. Angevine (1932), Lipid excretion, secretion of lipid into intestines J. Biol Chem 96, 769.

Spina (1889), Experimentelle Untersuchungen über die Bildung des Liquor *cerebrospinalis. Pflugers Arch. 76, 204.*

Spina (1900), Experimentelle Untersuchungen uber die Bildung des Liquor cerebrospinalis Pflugers Arch 80, 370

Spirow, M. S. (1927), Die Wege der Verteilung des Liquors und der injizierten Substanzen vom Subarachnoidalraum des Hirns und des Ruckenmarks. Russ Arch Anat. Hist. Embr.

(Spirow, M. S) *Спиров, М. С* (1937), Отводящие лимфатические сосуды щитовидной железы человека и их анатомические связи с отводящими лимфатическими сосудами дыхательной рубки Сб трудов, посв. 40-летию научн деятельн В Н. Шевкуненко.

(Spirow, M. S) *Спиров, М С.* (1953), Рецензия на книгу проф Жданова, Д. А. Общая анатомия и физиология лимфатической системы. Москва 1952

[illegible] erebrospinal fluid [illegible] ited lumbar punc-

[illegible] diabetica. Semana med 2, 231

Staehelin, R. (1925), Staubinhalation In: Handb. der normalen und patho- [illegible] Bethe u. G. Bergmann, vol II. Springer, Berlin

[illegible] Bronchien, der [illegible] nn—Staehelin.

[illegible] Bedeutung der [illegible] pathologischen [illegible]

Stahr, H (1900), Der Lymphapparat der Niere Arch. Anat. u. Physiol, Abt. Anat. 41

Standenath, *F. (1928), Das Bindegewebe. Seine Entwicklung, sein Bau und* seine Bedeutung für Physiologie und Pathologie. Ergebn. d Allg. Pathol. u. Pathol. Anat. 22, (II. Abt.), 70.

Starling, E. H (1893), Contribution on the physiology of lymph secretion. J Physiol. 14, 131

Starling, E. H. (1894), The influence of mechanical factors on lymphproduction. J. Physiol. 16, 224.

Starling, E. H. (1894/95), On the mode of action of lymphagogues. J. Physiol. 17, 30.

Starling, E. H. (1895/96), On the asserted effect of ligature of the portal lymphatics on the results of intravascular injection of peptone. J. Physiol. 19, 15

Starling, E. H. (1896 a), On the absorption of fluids from the connective tissue spaces. J. Physiol. 19, 312.

Starling, E. H. (1896 b), Physiological factors involved in the causation of dropsy. Lancet 1, 1267.

Starling, E. H. (1896 c), The causation of dropsy Lancet 1, 1407.

Starling, E. H. (1909), The fluids of the body. The Herter Lectures. W. T. Keener & Co , Chicago.

Starling, E. H. & A. H Tubby (1894), On absorption from and secretion into the serous cavities. J. Physiol 16, 140

S[illegible]
into t[illegible]
hand. [illegible]

S[illegible]
in no[illegible]
anoxaemia and fever. J Clin Investig 23, [illegible]

Stefanics, J., A. Balázs, L. Ránky & P Gorgő (1952), Az alsó végtag vénás trombózisa utáni késői panaszok (Late complaints following venous thrombosis of the lower extremity). Orv. Hetil (Hungarian), 93, 1385

(Shtefanis, F. A ) *Стефанис, Ф А.* (1902), О лимфатических сосудах почек человека, Киев

(Shtefko, V. G ) *Штефко В Г.* (1935), Патологическая анатомия лимфатических фаз туберкулезного процесса легких. Пробл. туберк. 9, 20

(Shtefko, V G ) *Штефко В Г* (1937), Вопросы патологической анатомии и клиники лимфатических фаз туберкулезного процесса легких. Пробл туберк 7

(Shtefko, V. G.) *Штефко В Г* (1938), Патологическая анатомия легочного и костного туберкулеза Москва

(Shtein, T. A.) *Штейн, Т А* (1948), К патологической анатомии очаговых пневмоний у детей Арх патологии 10, 75

Steinach, E & R H. Kahn (1903), Echte Contractilität und motorische Innervation der Blutcapillaren Pflugers Arch 97, 105.

Stenonis, N (1662), De insertione et valvula lactei thoracici et lymphaticorum Lugd Batav

Sterling, St (1911), Experimentelle Beiträge zur Pathogenese des Ikterus mit spezieller Berucksichtigung der Gallenkapillaren Arch Exper Path u Pharmakol 64, 468

Stewart, H J , N F Crane & J E Deitrick (1938), Absorption from the pericardial cavity in man Am Heart J 16, 198

Stilling, J (1887), Über die Lymphbahnen der Nebenniere. Virchows Arch 109, 324

Stohr, Ph (1909), Lehrbuch der Histologie. 13 Aufl Fischer, Jena

Storey, R H , J Mashman & J Furth (1951), A simple procedure for determination of the approximate lymph space Science 114, 665

Strauss, M B & H. J. Fox (1940), Anaemia and water retention Amer J. Med Sci 200, 454

(Strukov, A Y ) *Струков, А Й* (1933), Основные принципы чистоструктуры легкого Основы возрасной морфологии 9.

(Strukov, A Y ) *Струков, А Й* (1948), Формы легочного туберкулоза в морфологическом освещении.

Sugarman, J , M Friedman, E Barrett & T. Addis (1942), The distribution, flow, protein and urea content of renal lymph. Am. J Physiol 138, 108

Sullmann, H (1952), Über die Bestimmung von Dextran im biologischen Material. Helv Med Acta 19, 19.

Sumkow, I. (1847), Versuche die Lymphgefäße des Pericardium auf natürlichem Wege zu fullen Arch. ges Physiol. 8, 611.

(Sushko, A. A ) Сушко, А А (1950), Существует ли прямая связь между лимфатическими и венозными сосудами в органах. Сб. посв 50-летию научн деятельн А П Крымова, Киев.

(Sushko, A. A.) Сушко, А А. (1937), Морфологія відтікання лечeневої лімфи и людини. Експеримент Медицина Київ 1/19.

Suzuki, T. (1912), Zur Morphologie der Nierensekretion unter physiologischen und pathologischen Bedingungen. Fischer, Jena.

Swingle, W. W , W. Kleinberg & H. W Hays (1944a), Experimental analysis of nervous factor in shock induced by muscle trauma in normal dogs. Am. J Physiol. 141, 54.

Swingle, W. W., W. Kleinberg & H. W. Hays (1944b), Study of gelatin and saline plasma substitutes Am. J Physiol. 141, 329.

Swingle, W W., J. W. Remington, W Kleinberg, V. A Dril & W. J. Eversole (1942), Experimental study of tourniquet as method for inducing circulatory failure in dog. Am. J. Physiol. 138, 156.

Szabó, Gy. (1954), A nyirok képződését és a nyirokáramlást befolyásoló tényezők (Factors influencing lymphogenesis and lymph flow). Budapest Dissertation in Hungarian.

Szabó, Gy. (1955), A nyirokkeringés *physiologiai és pathologiai jelentősége* [illegible] f lymph circulation) Magy Belorv.

[illegible] árton & K. Tornyos (1956), Wirkung Blutvolumens auf die renale Salz- Menschen und bei dekompensierten

[illegible] ibó & Zs. Magyar, Die Wirkung von Histamin auf die Kapillarpermeabilität.

Szabó Gy & I Littmann (1952), A portális hypertonia sebészi kezelése (Surgical treatment of portal hypertonia). Magyar Belorv Arch. (Hungarian), 5, 118

Szabó Gy. & Zs Magyar (1954), A glomerulus filtratio mérése dextrannal (Measurement of glomerular filtration with dextran) Kísérl Orvostud (Hungarian), 6, 197.

Szabó Gy. & Zs. Magyar (1956a), Capillaris permeabilitás és nyirokáramlás ischaemiás shockban (Capillary permeability and lymph flow in ischaemic shock) MTA V. Oszt Közl. (Hungarian), 7, 23.

Szabó, G. & Zs. Magyar (1956b), Perméabilité capillaire en état de choc. Semaine des hôp

Szabó, Gy. & Zs. Magyar (1956c), Die Wirkung des Histamins auf die Kapillarpermeabilität. Acta Med. Hung. 10, 55

[illegible] sche [illegible] sra (Eff [illegible] n). [illegible] íra (Eff[illegible] zl. (Hungarian), 8, 281.

Szabó, Gy., J. Rév & F. Solti (1956a), Humorális tényezők szerepe az effective keringő vérvolumen változásai hatására létrejövő víz- és sóürítés változásokra (Role of humoral factors in the changes of water and salt excretion caused by changes in the volume of actually circulating blood). Magy. Belorv. Arch (Hungarian), 9, 92.

Szabó, Gy., J. Rév & F Solti (1956b), Über die Rolle humoraler Faktoren bei den durch Veränderungen des effektiven kreisenden Blutvolumens zustandekommenden Abweichungen in der Wasser- und Salzausscheidung. Acta Med Hung. 10, 15

Szinay, Gy & J. Szeker (1955), A gyomorfal chronicus granulomás lymphangitise (Chronische Lymphangitis mit Granulom der Magenwand). Kísérl. Orvostud 7, 333.

Szontágh, F. (1948), Adatok az intracutan befecskendezett physiologiás NaCl-oldat felszívódási idejének (quaddli próba) jelentőségéhez (Data concerning the significance of the time of absorption of intracutaneously introduced physiol. saline). Orv. Hetil. (Hungarian), 89, 57.

Takahashi, S. (1933), Ueber die Antikorperbildung in den Lymphdrüsen. Arb III. Abt Anat. Inst. Kaiserl. Univ Kyoto Ser D. 3, 67.

Takáts, G. & M H. Evoy (1950), Lymphedema. Angiology 1, 73.

Talalajeff, W. (1927), Beiträge zur Frage von der Lymphostase. Virchows Arch. 266, 268.

Tamáska, L & L. Harsányi (1955), Über die periarteriellen Lymphspalten der Lunge. Acta Morph. Hung 6, 45.

Tani, I. (1924), Untersuchungen zur Permeabilität der Zellen XI. Untersuchungen uber den Einfluß des gesteigerten Blutdruckes auf den Flüssigkeitsaustausch zwischen Blut und Gewebe. Biochem Z. 145, 189.

Tanos, B. (1954), Ugynevezett „hialuronidáz" készítmények komplex természetéről (On the complex nature of so-called "hyaluronidase" preparations) Lecture held in Hungarian.

Tanos, B, E Kelemen & R. Soltész (1953), Die Rolle der Nebennierensysteme in den sogenannten Antihyaluronidasewirkungen Acta Med. Hung 4, 419

Tattoni, G & A. Mazelle (1952), Le reti linfatiche della cistifellea nella colelitiase. Giorn. Ital. Chirurg. 8, 714

Taylor, G. W, J. B. Kinmonth, E. Rollinson & J. Rotblat (1957), Lymphatic circulation studied with radio-active plasma protein Brit Med J p. 133.

Teichman, L. (1861), Das Saugadersystem vom anatomischen Standpunkte. Engelmann, Leipzig

Tendeloo, N. Ph. (1902), Studien über die Ursachen der Lungenkrankheiten. Bergmann, Wiesbaden

Tendeloo, N. Ph (1925), Formen, Fälle und Verlauf der Lungentuberkulose [illegible]

[illegible] Москва, 50

Terry, R., D R Hawkins, E H. Church & G. W Whipple (1948), Proteinuria related to hyperproteinaemia in dogs following plasma given parenterally A renal threshold for plasma proteins J. Exp Med 87, 561

Testut, J. L (1894), Traité d'anatomie humaine, anatomie descriptive, histo- [illegible]

[illegible] tok a vagus-pneumonia pathogenesisével [illegible] of vagus-pneumonia) Szeged Lecture

[illegible] (1896) Об окончании нервов в мужских полевых органах у человека и млекопитающих Казань Ref.. Ergebn d Anat. u Entwicklungsgesch 7, 627 (1897)

Tocker, A M & H T Langstjon (1952), The perivascular space of the pulmonary vessels. J. Thorac Surg. 23, 539

Tomasewsky, Z. & G G. Wilenko (1908), Beitrag zur Kenntnis der antagonistischen Wirkung des Adrenalins und der Lymphagoga Berl. Klin. Wschr 1, 221.

Tomsa, W (1863), Die Lymphwege der Milz. Sitzgsber Akad Wiss Wien Math -naturwiss Cl 48, 652

Tonkoff, W. (1899), Zur Kenntnis der Nerven der Lymphdrusen Anat Anzeiger 16, 456.

Town, B W., E. D. Wills u. A. Wormall (1949), Action of suramin and "antrycide" on enzymes. Nature. 164, 233.

Tretjakoff, D. (1926), Die orbitalen Venensinusse der niederen Wirbeltiere Morphologisches Jahrb 56, 402.

(Troitskaya, A. A ) *Троицкая, А. А* (1946), Лимфатические сосуды диафрагмы Вести хир. и погран. обл 6/16, 35.

Tscherewkow, A. (1896), Einige Versuche uber den Einfluß von Blutentzieh ... Pflugers Arch 62, 304

... stickten

T

... hstroms

u

... deration

Proc 13, 154.

Uljanov, P. N (1929), Zur Frage der Verbindungen zwischen den subarachnoidalen Räumen des Gehirns und dem Lymphsystem des Korpers. Zeitschr. ges exp Med. 65, 621.

Unghváry, L. (1948), Die EKG-Diagnose der venosen Zirkulationsstorung des Herzens. Schweiz. Med. Wschr. 78, 901.

Unna, P. (1908), Untersuchungen uber die Lymph- und Blutgefäße der äußeren Haut ... Haarfollikels. Leipzig. Med. Diss.

... zur Kenntnis der physiologischen Wirk ... u. Pharmakol. 2, 89

... рлексы а внутренних органов и сосудов на желче- и лимфоотделение. Труды Всес. Общ. Физиол , биохим, фармакол. 2, 64

Ussing, H. H (1948), The use of tracers in the study of active ion transport across animal membranes Cold Spring Harbor Symp. Quant. Biol 13, 193.

Ussing, H. H (1949), The distinction by means of tracers between active transport and diffusion Acta physiol Scand. 19, 43.

(Valeyeva, Z. T.) *Валеева, З. Т.* (1948 а), Об иннервации грудного протока и реакции его на некоторые яды Фарм. и токс 11/5, 36.

(Valeyeva, Z. T.) *Валеева, З. Т.* (1948 б), К вопросу иннервации грудного лимфатического протока собаки и реакции его на некоторые фармакологические вещества Уфа Thesis

(Valeyeva, Z. T.) *Валеева, З. Т* (1949), Рефлекторные влияния с грудного лимфатического протока на кровеносную систему. Сборн научн труд Башкирск Мед ин-та Уфа.

(Valeyeva, Z. T ) *Валеева, З. Т* (1954), О рецепторах cisternae chyli. Тр Всес Общ Физиол биох. Фармакол 2, 67.

cortic

la ly

92, 405.

Vanucci, ... 77, 425.

Vargha, ...

stitialis tüdő

Orv. Hetil (Hungarian), 9?, 1??0

Vaughan, J. H., A. Armato, J. Goldthwaite, P. Brachman, C B. Favour & T. B. Bayles (1952), Fate of intravenously injected labelled gamma globulin in the normal subject and in patients with rheumatoid arthritis J. Clin Investig 31, 668.

Várady, K. (1947), Radiographie du système lymphatique de l'utérus. Schweiz. Med. Wschr. 18, 509.

Várady, M. K. (1951), Vitesse de propagation dans les lymphatiques utérins. Gynécologie et Obstétrique 50, 207.

Vecchi, A. (1911), Die anatomischen Grundlagen der Chirurgie der Lymphdrüsen: die Regeneration und Neubildung. Mitteil. Grenzgeb. Medizin u. Chir 23, 12.

(Velikoretshin, I. A.) *Великоречин, И. А.* (1949), Слияния и анастомозы лимфатический сосудов желудка и поперечной ободочной кишки. Труды Ленингр. сан.-гиг. мед. ин-та 3, 119.

Vendég, V. (1948), A submucosus septum resectio alkalmával fellépő rosszullétek [szédülés, verejtékezés, eszméletvesztés] magyarázata (Explanation of vertigo, perspiration and syncope following resection of the submucous septum). Kolozsvár. (Hungarian).

Verzár, F. (1929), Die Resorption aus dem Darm. In: Handb. der normalen u. path. Physiol. hrsg. A. Bethe u. G. Bergman. Springer, Berlin.

Végh, L., L. Kocsár, & L. Kertész (1957), Isotopic studies of the pleural lymphatic circulation. Acta Med. Acad. Sc. Hung. 10, 233.

Végh, L., L. Kocsár & L. Kertész (1958), Untersuchung des Lymphkreislaufs der Leber mit Isotopen im Organschock. Acta Med. 11, 397.

Vialleton, L. (1903), Lymphatiques valvulés et ganglions lymphatiques. Bibl. Anat. 12, 19.

Vierth (1895), Über rückläufige Metastase in den Lymphbahnen. Zieglers Beitr. 18, 515.

Vieussens, R. (1705), Novum vasorum corporis humani systema. Amstelod.

Vimtrup, B. (1923), Beiträge zur Anatomie der Capillaren. II. Weitere Untersuchungen über contractile Elemente in der Gefäßwand der Blutcapillaren. Z. ges. Anat. I. Abt. 68, 469.

Virchow, R. (1848), Zur Diagnose des Krebses im Unterleibe. Med. Reform 248.

Virchow, R. (1851), Cellularpathologie. Berlin.

(Visotski, N. F.) *Высоцкий, Н. Ф.* (1877), Лимфатическая система обваливающих апоневрозов. Уч. зап. Каз. ун-та 3.

(Vitels, L. G.) *Вительс, Л. Г.* (1947), Кровеносные и лимфатические сосуды реберной плевры человека. Тез. докл. 1 сесс. Ин-та морфол. АМН СССР 6.

Vogel, L. (1891), Über die Bedeutung der retrograden Metastase innerhalb der Lymphbahn für die Kenntnis des Lymphgefäßsystems der parenchymatösen Organe. Virchows Arch. 125, 495.

Vogt, M. (1944), Observations on some conditions affecting rate of hormone output by suprarenal cortex. J. Physiol. 103, 317.

Volhard, F. (1931), Nieren und ableitende Harnwege. In: Mohr—Staehelin, Handb. d. inn. Med. VI/1. 2nd ed. Springer, Berlin.

Volkmann, A. W. (1844), Nachweisung der Nervencentra, von welchen die Bewegung der Lymph- und Blutgefäßherzen ausgeht. Müller's Arch. Anat. Physiol. 418.

Volkmann, A. W. (1874), Untersuchungen über das Mengenverhältnis des Wassers und der Grundstoffe des menschlichen Körpers. Verh. sächs. Ges. Wiss. Leipzig, Math.-Phys. Kl. 26, 202.

Volwiler, W. (1951), The relation of certain mechanical factors to the production of ascites. In: Liver disease. Ciba Foundation Symposium. London.

Volwiler, W., J. L. Bollman & J. H. Grindlay (1950a), A comparison of two types of experimental ascites. Proc. Staff Meet. Mayo Clin. 25, 31.

Volwiler, W., J. H. Grindlay & J. L. Bollman (1950b), The relation of portal vein pressure to the formation of ascites. — An experimental study. Gastroenterology 14, 40.

Volwiler, W., K. Fremont-Smith & M. P. MacMartin (1952), Turn-over rates of serum proteins in cirrhotic man. J. Clin. Investig. 31, 668.

(Vrublevski, F. J.) *Врублевский, Ф. И.* (1942), Лимфатические сосуды околосердечной сумки. Сб. «Материалы к анатомии лимфатический сосудов и узлов». Горький.

(Vyodze, D.) *Выводуев, Д* (1865), О лимфатических сосудах легкий. Санкт-Петербург.
Wywodzew, D. (1866), Die Lymphwege der Lunge. Wiener Med. Jahrb 11
(Wachtel, V. S.) *Вахтель, В. С* (1946), О лимфообращении после экстирпации лимфатических узлов. Хирургия 3, 16.
(Wachtel, V. S.) *Вахтель, В С* (1955), Рентгенологическое изучение лимфообращения при отморожениях и ожогах. Хирургия 9, 54.
Wadsworth, T. W. (1922), A mechanism maintaining the negative pressure of the pleura and influencing absorption. J. Path. and Bact. 25, 266.
Wakai H & Y. Makoto (1935), Pharmakologie des Lymphherzens. I. Mitt. Fol Pharmacol. Jap. 21, 70.
Wald, B & V. Varteresz (1948), Experimental studies on antiphlogistic effects of roentgen rays. Radiol. Clin. 17, 348.
Waldeyer, W. (18( ... lungen uber die Lymphherzen der F ...
Walker, A. M., P. ... The collection and analysis of fluid from single nephrons of the mammalian kidney. Am. J. Physiol 134, 580
Walther, A. F. (1731), De ductu thoracico bipartito, Vena bronchiali sinistra et inferiore arteria hepaticae, superioris mesaraicae sobole. Lipsiae
Warner, W. T. & J. A. Dauphinée (1936), Thrombosis of coronary venous sinus in case of thrombophlebitis migrans. Amer. Heart J. 12, 483
Warren, M F. & C K Drinker (1942), The flow of lymph from the lungs of the dog Am J. Physiol. 136, 207
Warren, M. F , D K. Peterson & C. K. Drinker (1942), The effects of heightened negativ pressure in the chest, together with further experiments upon anoxia in increasing the flow of lung lymph. Am. J. Physiol 137, 641.
Warren, S. & S C. Sommers (1948), Cicatrizing enteritis (regional ileitis) as a pathologic entity Analysis of one hundred and twenty cases. Am J. Path 24, 475
Wasserman, K & H. S. Mayerson (1951), Exchange between plasma and lymph. Am J Physiol. 165, 15
Wasserman, K & H. S. Mayerson (1952 a), Mechanism of plasma protein changes following saline infusions. Am. J Physiol 170, 1.
Wasserman, K. & H. S. Mayerson (1952 b), Plasma, lymph and urine studies after dextran infusion. Am J Physiol 171, 218
Wasserman, K. & H. S. Mayerson (1952 c), Dynamics of lymph and plasma protein exchange. Cardiologia 21, 296
Watkins, A. L & M N. Fulton (1938), The effect of fluids given intraperitoneally, intravenously and by mouth on the volume of thoracic duct lymph in dogs. Am. J. Physiol 122, 281.
Wearn, J T & A. N. Richards (1924), Observations on the composition of glomerular urine, with particular reference to the problem of reabsorption in the renal tubules Am J Physiol. 71, 209
Webb, R L (1933), Observations on the propulsion of the lymph through the mesenteric lymphatic vessels of the living rat. Anat. Record 57, 345
Webb, R L. (1937), Mesenteric lymphatics. Analysis of the mechanism of drug action as revealed by motion pictures. Proc. Inst. Med Chicago. 11, 15
Webb, R. L (1952), The lymphatic system. Ann. Rev. Physiol 14, 315.
...
subcutaneous lymph in dogs. J. Clin Invest. 12, 1021.
... and composi-
... 63.
... a in the dog
... 50, 75.

Weed, L. W. (1914), Studies on the cerebrospinal fluid III. The pathways of escape from the subarachnoid spaces with particular reference to the arachnoid villi. J. Med. Res. 31, 31.

Weed, L. W. (1922/23), The absorption of cerebrospinal fluid into the venous system. Amer. J. Anat. 31, 191.

Weese, H. & W. Scholtan (1951), Pharmakologie des Periston N., Dtsch. Med. Wschr. 76, 1187.

Wegner, G. (1877), Chirurgische Bemerkungen über die Peritonealhöhle mit besonderer Berücksichtigung der Ovariotomie Arch. klin. Chir. 20, 51.

Weil, R. (1917), The vasomotor depression in canine anaphylaxis., J Immunol. 2, 429.

Weinstein, L. (1940), Further studies on prophylaxis of experimental infections ... preparations, Yale J Biol, Med. 12, 519.

... vena aqueosán (Experiments on the V. ... 29.

... орфологиа стенки грудного протока и

... congestion of lungs resulting from intra-

Weiss, J. (1894), Beitrag zur Kasuistik des Ascites chylosus. Centralbl. Inn Med. 15, 665

Weiss ... Path. An. ...

Welc ...

Webb ... u. Siredon ...

Welіky, W. (1886), Ueber die Lymphherzen bei Siredon pisciformis Mélanges biol. Acad. imp. d sc. St Petersb. 12, 311.

Wells, B B & E C Kendall (1940), A quantitative difference in the effectivity of compounds separated from the adrenal cortex on distribution of electrolytes and on atrophy of the adrenal and thymus glands of rats Proc Staff Meet Mayo Clin 15, 133.

Wells, H S (1932), The concentration and osmotic pressure of the proteins in blood serum and lymph from the lacteals of dogs Am J Physiol. 101, 421

Wells, H S, G B. G Youmans & D. G. Miller Jr. (1938), Tissue pressure (intr... capil... ektor...

Wertheimer, E (1900), Travail des glandes et lymphogenèse Journ Physiol. Pathol. Gén. 8, 801

Wertheimer, E. (1923), Über irreziproke Permeabilität. III. Mitt. Die irreziproke Permeabilität von Ionen und von Farbstoffen Pflugers Arch., 200, 354.

Wertheimer, E. & L. Lepage (1897), Sur les voies d'absorption des pigments ...

... de la

... Buda-

Westcott, R N., N O Fowler, R. C Scott, V. D. Hauenstein & J. McGuire (1951), Anoxia and pulmonary vascular resistance J. Clin Investigation. 30, 957.

Whipple, G H. u. J. H. King (1911), The pathogenesis of icterus. J Exp. Med. 13, 115

White, A. & T. F. Dougherty (1945), The pituitary adrenocorticotrophic hormone control of the rate of release of serum globulins from lymphoid tissue. Endocrinology. 36, 207.

White, H. L (1929), Observations on the nature of glomerular activity. Am. J Physiol. 90, 689.

White, J. C., M E. Field & C. K. Drinker (1933), On the protein content and normal flow of lymph from the foot of the dog. Am. J. Physiol 103, 34

White, J. C. & S. Warren (1944), Causes of pain in feet after prolonged immersion in cold water. War. Med. 5, 6.

Wiggers, C. J. (1950), Physiology of shock. Commonwealth Found. London

Wiggers, C. J , D. F. Opdyke & J. R. Johnson (1946), Portal pressure gradients under experimental conditions, including hemorrhagic shock Am. J. Physiol 146, 192.

Wiggers, H. C., R. C. Ingraham, F. Roemhild & H. Goldberg (1948), Vasoconstriction and development of irreversible hemorrhagic shock Am. J Physiol. 153, 511.

Wilhelm, D. L., A. A. Miles & M. E. MacKay (1955), Enzyme-like globulins from serum-reproducing vascular phenomena of inflammation: isolation and properties of permeability factor and its inhibitor. Brit. J. Exp. Path. 36, 82

Willis, T. (1675), Pharmaceutica rationalis

Wilson, F. N., J. G. W. Hill & F. D. Joh [illegible] electrocardio- [illegible] of the cat. Bull. John Hopkins Hosp. 41, 226.

Winter, R (1944/47), Seröse Entzundung und pericapillares Ödem der Leber. Beitr pathol. Anat 109, 480.

W [illegible] function. Trans [illegible]

[illegible] erimental pneu [illegible]

Winterstein, H. (1925), Handbuch der vergleichenden Physiologie Vol. I. (I. Hälfte.) Jena. Fischer.

Wintrobe, M. M & M. V. Buell (1933), Hyperproteinaemia associated with multiple myeloma. Bull. John Hopkins Hosp. 32, 156.

Wischnewsky, A S (1928), Über die Bedingungen einer verschiedenen Schnelligkeit der Fortbewegung von Farbstoffen im Nerv. Ztschr. ges exp Medizin. 61, 107.

Wiseman, B. K. (1931), The induction of lymphocytosis and lymphatic hyperplasia by means of parenterally administered protein J. Exp Med. 53, 499.

Wislocki, G. B (1916/17), The staining of amphibion larvae with benzidine dyes with especial reference to the behaviour of the lymphatic endothelium. Am. J. Physiol. 42, 124.

With, T. K (1947), The pathogenesis and different forms of jaundice. Acta Med Scand 128, 25.

With, T K. (1949), On jaundice. Acta Med Scand. Suppl 234, 331.

Wittich (1874), Über die Lymphbahnen in der Leber, Zentralbl. med Wissenschaft. 12, 914.

W [illegible] the heart-lung [illegible] eus und die [illegible] experimen- [illegible] ningozellen- [illegible]

Wutzer, C. W. (1834), Einmundung des Ductus thoracicus in die Vena azygos. Müllers Arch. 311.

Yamada, S. & al. (1933), Über die seröse Flussigkeit in der Pleurahohle der gesunden Menschen. Z. ges. exp Med 90, 342.

Yamamoto, I. (1924) [illegible] ...schen [illegible]

[illegible] при так называемых хронических неспецифических пневмониях. Арх. патологии 14/2, 34.

Yoffey, J. M. (1932/33), The quantitative study of lymphocyte production J. Anat. 67, 250.

Yoffey, J. M. (1935/36), Variation in lymphocyte production. J. Anat. 70, 507.

Yoffey, J. M. (1939 a), Nasal mucous membrane in relation to lymph stream and cerebrospinal fluid. Proc. Roy. Soc. Med. 41, 798, 1948 und J. Laryng and Otol. 63, 166.

Yoffey, J. M (1939 b), Nose in relation to cerebrospinal fluid and lymph stream Arch. Dis. Childhood. 24, 117.

[illegible] and [illegible] atic apparatus. J. Exp. Med. 70, 83.

Yoffey, J. M. & E. R. Sullivan (1939), Lymphatic pathway from nose and pharynx; dissemination of nasally instilled vaccinia virus. J. Exp. Med 69, 133.

Yoffey [illegible] extract of [illegible]

Zamb [illegible] da arsenico. [illegible] 32, 402

(Zaverkin [illegible] (1863), Корни лимфатических сосудов в по[illegible] Воен мед. ж 6, 169.

[illegible] Fettstromes durch den Brustgang nach [illegible] g 11, 147.

Zeckwer, I. T. (1947), Limitation of spreading factor in injured rats Arch. Path. 44, 356.

Zehnder, (1890), Über regenerative Neubildung der Lymphdrusen Virchows Arch. 120, 294

(Zhdanov, D. A.) Жданов, Д А (1934) О некоторых хистофизиологических особенностях стенки лимфатических сосудов Труды I Всеросс гистол. конф. 207.

(Zhdanov, D. A.) Жданов, Д А (1940a), Функциональная анатомия лимфатической системы Изд. Горьковск мед ин-та Горький.

(Zhdanov, D A.) Жданов, Д А (1942b), Пути всасывания из плевральных полостей. Пробл туберк 11, 56

(Zhdanov, D A.) Жданов, Д А (1942a) Об участии кровеносных и лимфатических сосудов сальника в резорбции истинных и коллоидных растворов и взвесей из брюшной полости Сб. «Материалы к анатомии лимфатических сосудов и узлов» Горький

(Zhdanov, D A ) Жданов, Д А (1945), Анатомия грудного протока и главных лимфатических коллекторов туловища. Труды Томского мед ин-та 14, 3.

(Zhdanov, D. A.) Жданов, Д А (1946), Пути всасывания растворов и взвесей из серозных полостей Сб «Вопросы хирургии войны и абдоминальной хирургии».

(Zhdanov, D. A ) Жданов, Д А (1948), К анатомии лимфатических сосудов пищевода Сб. трудов посв проф А Г Савиных Томск

(Zhdanov, D. A.) Жданов Д А. (1949a), О связях лимфатической системы желудка [illegible] ов. Врач. дело 1015.

(Zhda[illegible] ии исследования главных лимфатич[illegible] д. ин-та 3, 14.

(Zhd[illegible] анные к анатомии лимфатической [illegible] сан-гиг. мед. ин-та 9, 5.

(Zhd[illegible] анатомия и физиология лимфатической системы. Медгиз, Ленинград.

(Zhdanov, D. A.) *Жданов Д. А.* (1953a), Анастомозы и слияния лимфатической системы желудка и двенадцатиперстной кишки человека. Труды Ленингр. сан.-гиг. мед ин-та **17**, 103.

(Zhdanov, D. A.) *Жданов Д А.* (1953b), Об отношении периневральных пространств вегетативных нервных сплетений кишечной трубки и лимфатической системе. Труды Ленингр. сан -гиг мед. ин-та **2**, **17**, 112.

(Zhdanov, D. A.) *Жданов, Д А.* (1955), Новые данные к анатомии интраорганной лимфатической системы внутренностей. Арх. анат. гистол. и эмбриол 32/3, 28.

(Zhdanov, D. A. & S. S Pavlitskaya) *Жданов, Д. А.* и *С. С. Павлицкая* (1949), К иннервации стенки грудного протока Труды Ленингр сан -гиг мед ин-та **2**, 5.

(Zhemtchuzhnikova, L. Y ) *Жемчужникова, Л. Й* (1953), Лимфатическая система сердца человека. Труды Ленингр. сан.-гиг. мед. ин-та **17**, 63.

Zielinska, M. (1894), Beiträge zur Kenntnis der normalen und strumosen Schilddruse des Menschen und des Hundes Virchows Arch. **136**, 170.

Zimmerman, L. H. & G Takáts (1931), The mechanism of thrombophlebitic edema. Arch. Surg. **23**, 937.

Zollinger, H. U. (1951), Die pathologische Anatomie der Nephritiden. Helv. Med. Acta **18**, 269.

Zothe, H. (1942), Zur Pathogenese des Ödems. Dtsch. Arch. klin. Med. **189**, 253.

Zschau, H. (1933), Untersuchungen über das Lymphgefaßsystem des großen Netzes. Dtsch. Ztschr. Chir. **240**, 395.

Zweifach, B. W. (1936/37), The structure and reactions of the small blood vessels in amphibia. Am J Anat. **60**, 473.

Zweifach, B. W. (1939), The character and distribution of the blood capillaries. Anat. Rec. **73**, 475.

Zweifach, B. W. (1950), In Ground Substance: Trans. 1st Conf., J. Macy Found. New York.

Zweifach, B. W., S Baez & E. Shorr (1952), Effect of dibenamine on humoral and vascular changes in dogs subjected to hemorrhagic shock. *Federation Proc.* **11**, 79

Zwemer, R. L. & C. Lyons (1928), Leukocyte changes after adrenal removal Am. J. Physiol. **85**, 545.

## SOVIET AND RUSSIAN AUTHORS*

Andreyev, F. A. (1939), Lungs and pleural cavity. Collected works to the 15 years anniversary of the Belorussian State Institute of Medicine 73.

Arustamova, A. T. (1938), Tuberculosis of bronchial lymph glands of adults. "Problemy tuberk." 4, 18.

Braude, A. I. (1948), The role of mesothelium in the reabsorption from of the abdominal cavity. "Byull. eksp biol. med" 24, 222.

Braude, A. I. (1950), The role of the large uterine ligaments in the formation of peritoneal effusions "Byull eksp. biol. med." 27, 143.

Drozdova, A. V. (1953), Collateral lymph circulation in the small intestines under conditions of incomplete desympathization and deafferentation "Arh anat. gist. i embriol." 30/4, 63.

[illegible] cosis "Silikoz. trud [illegible] nodes by the crem

Frautchi, V. H. (1938), Anatomical analysis of lymphohematogenic metastases "Vopr. klinicheskoy i eksperimentalnoy onkologii" 104

Frautchi, V. H. (1948), Lymphovenous anastomoses in man. "Hirurgiya" 11, 12.

Goldstein, D. E (1950), Materials for the study of the lymph system by method of *in vivo* lymphography. Kazan 1949. "Sov. ref. obozr." 3/35.

* The original works see in the bibliography p. 749.

Gryaznova, A. V. (1953), Are there, in man, lymphovenous anastomoses between the thoracic duct and the azygous and hemiazygous veins? "Arh. anat. gist. i embriol." 30/2.

Gryaznova, A. V. (1955), The problem of lymphovenous anastomoses between the thoracic duct and the azygous veins in dog "Arh anat. gist. i embriol." 32/3, 93.

Ivanov, G. F. (1936), Some forms of the lymph circulation outside the vessels. "Arh biol. nauk." 48, 214.

Ivanov, G. F. (1939), About the intra-adventitial systems of bronchi. "Arh. biol. nauk", 53, 123.

Ivanov, G. F. (1953), To the critique of some theories about thee pathways of lymph out-flow. "Arh. anat. gist. i embriol." 30/5, 78

Kokhanina, M. Y. (1948), Influence of painful reflexes on the lymph-flow. "Vestn. AN Kazahskoy SSR" 45/12, 95

Kokhanina, M. Y. *(1949a), Reflexes from pressoreceptors of the intestinal,* renal and renal pelvis vessels on the lymph-flow "Izv AN Kazahskoy SSR, ser. fiziolog" 73/2, 111.

Kokhanina, M. Y. (1949b), Reflex-humoral regulation of the lymph-flow. "Izv. AN Kazahskoy SSR, ser fiziolog" 73/2, 111

Kokhanina, M. Y. (1951), Reflexes from the spleen on the lymph-flow. "Sov. med ref. obozr." 8, 43.

Kossitsyn, I. I. (1946), Development of the lymphatic system of the thyroid gland in man. "Sb. trudov Izhevsk. gos. med. in-ta" 5, 181.

Kossitsyn, I. I. (1946), About the possibility of the inflow of lymph vessels into the veins without interruption, in lymph nodes. "Sb. trudov Izhevsk. gos. med. in-ta"5, 175.

Kossitsyn, I. I. (1953), Nerve apparatus of the lymph nodes. "Vopr. morfologii" 2, 190.

Kovanov, K. V. (1954), The lymph system as a regulator of the blood pressure "Trudy Vses. obshch. fiziol. biohim. i farmak." Moscow.

Kudrin, I S. (1953), To the anatomy of the lymph system of the uterus. "Trudy Leningrad. san. — gig med. in-ta" 17, 186.

Mamiyev, S T. (1953), The lymph vessels of the trachea of man "Trudy Leningrad. san -gig. med in-ta" 17, 5

Matotchkin, I. N (1949), The lymph vessels of the diaphragm and their absorptive capacity. "Sb. trudov Arhang med. in-ta" 9, 89.

Melnikov, A. V. (1945), About the cancer of the stomach. (A clinical study.) Leningrad.

Minkina, N A (1953), The lymph system of the human costal and diaphragmatic pleura. "Trudy Leningr. san -gig med. in-ta" 17, 47.

Mogilnitsky, B N (1949), The problems of the permeability of blood capillaries in pathology. "Medgiz", Moscow.

Nadezhdin, V N (1951), Architecture of the end lymphatic network of the skin in human lower extremities "Trudy Leningr. san.-gig. med in-ta" 9, 115.

Nalintsev, M E (1951), The lymph vessels of the skin of human body. "Trudy Leningr. san -gig. med. in-ta" 9, 97.

Norenberg-Tcharkviani, A. E (1954), Acute appendicitis as seen in the light of the modern pathogenetic doctrine "Klin. med" 5/22.

Okun, N. S (1943), Lymph. circulation in infections. "Vest hir." 63, 27.

Okunev (Okuneff) N V. (1939), The problem of permeability in pathology. *"Problemy pronitsayemosti"*, Moscow.

Oleandrov, L V (1939), Pathologic alterations of permeability. "Problemy pronitsayemosti", Moscow.

Parfenova, I P. (1950), Lymphatic network of the pulmonary lobules. "Dokl. AN SSSR" 72, 613

Parfenova, I P. (1952), The lymphatic system of the normal lung in anatomo-roentgenologic reflection. "Probl tuberk." 1, 20

Petrovski, V V. (1954), The role of the lymph vessels in blood circulation. "Fiziol zhurn SSSR" 40, 322.

Pozharski, P., N. Toltchskaya & A. Shilova (1950), Pathological anatomy of the silicosis. "Arh. patologii" 2, 13.

syste

pleura and lung in advanced age. Probl. tuberk. 9, 40.

Ravitch-Shtcherbo, V. A. (1940), On the physiological retrograde lymph-flow of the endothoracic area. "Sb. Retrogradny limfotok endotorakalnoy oblasti" Voronezh.

Reingold, M. L. (1944), To the problem of the retrograde mediastinocardinal flow of the lymph. "Sb. Retrogradny *limfotok endotorakalnoy* oblasti." Voronezh.

Rindovski, D. F. (1871), The lymph vessels of the kidneys. "Trudy III cyezda russkih yestestvoispytateley. Otd. nauchn. med." 57.

Rotenberg, A. L. (1949), Confluence of efferent lymph vessels of liver and stomach. "Trudy Leningr. san.-gig. med. in-ta" 3, 160.

Rotenberg, A. L. (1952), Intra-organic lymphatic system of the lung "Trudy Leningr. san.-gig. med. in-ta" 17, 14.

Rusznyák, I. (1954), New researches in the physiology and pathology of the lymph circulation. "Klinich. Medits." 32/1, 23.

Shaklein, I. A (1950), Significance of the pulmonary lymphatic system at healing pneumothorax. "Probl. tuberk." 1, 31.

Stefanis, F. A. (1902), On the lymph vessels of the human kidneys. Kiev.

Shtefko, V. G. (1935), Pathological anatomy of the lymphatic phases of tuberculous processes of the lung. "Probl. tuberk." 9, 20.

Shtefko, V. G. (1937), Problems of pathological anatomy and clinics of the lymphatic phases of tuberculous processes of the lung. "Probl. tuberk" 7.

Shtefko, V. G. (1938), Pathological anatomy of pulmonary tuberculosis and of tuberculosis of bones and joints. Moscow.

*Sikorski, N. (1872), On the lymph vessels of the lung. Kiev.*

Skvortsov, Y. Y (1874), Materials to the anatomy and histology of the pericardium of man. Saint Petersburg.

thyroid gland and the trachea. "Sb.

General anatomy

. Arhiv. anatomii,

al anatomy of the lobular pneumonia of children. "Arh. patologii" 10, 75.

Strukov, A. Y. (1933), Fundamental principles of the histostructure of the lung. "Osnovy vozrastnoj morfologii" 9.

Strukov, A. Y. (1948), Forms of pulmonary tuberculosis in the light of the morphology.

Sushko, A. A. (1937), Morphology of lymph flow in man. "Eksperiment Meditsina Kiev" 1/19.

Sushko, A. A. (1950), Are there direct communications between the lymphatic and venous vessels in the organs? "Sb. posv. 50-letiyu nauchn. deyateln A. P. Krymova, Kiev."

vessels of the human kidneys and

ations of the liver in Botkin's dise

ment. "Pat. anat inf bol." Moscow 50

Timofeyev, D. A. (1896), On the nerve endings in the male genital organs of man and mammalia. Kazan.

Troitskaya, A. A. (1946), The lymph vessels of diaphragm. "Vestn. hir. i pogran obl." 6/16, 35.

[illegible]
and l [illegible]
react [illegible]
thors [illegible]
Ufa. Dissertation.

Valeyeva, Z. T. (1949), Reflex influences from the thoracic lymph duct on the blood circulation system. "Sbor. nauchn. trud. Bashkirsk. Med. in-ta." Ufa.

Valeyeva, Z. T. (1954), About the receptors of the Cisterna chyli. "Tr. Vses. Obshch. Fiziol. biohim. Farmakol." 2, 67.

Velikoretchin, I. A. (1949), Confluences and anastomoses of the lymph vessels of stomach and transverse colon. "Trudy Leningr. san.-gig. med. in-ta" 3, 149.

Visotski, N. F. (1877), The lymphatic system of the aponeuroses. "Uch. izv. Kaz. in-ta" 3.

Vitels, L. G. (1947), Blood and lymph vessels of the human costal pleura. "Tez. dokl. I sess. In-ta morfol. AMN SSSR" 6.

Vrublevski, F. I. (1942), The lymph vessels of the pericardium. "Sb. Materialy k anatomii limfaticheskih sosudov i uzlov." Gorky.

Vyvodzev (Wywodzew) D (1865), About the lymph vessels of the lung. Saint Petersburg.

Wachtel (Vahtel), V. S. (1946), About the lymph circulation after extirpation of lymph nodes. "Hirurgiya" 3, 16.

Wachtel (Vahtel), V. S. (1955), Roentgenological analysis of the lymph circulation in case of congelation and combustion. "Hirurgiya" 9, 54.

Weirich (Veyrih), G. (1851), The morphology of the walls of the thoracic duct and of other lymph vessels. Yurev (Tartu).

Yessipova, I. K. (1952), Contributions to the pathogenesis of the pneumosclerosis in case of so-called chronic nonspecific pneumonia. "Arh patologii" 14/2, 34.

Zavarykin (Savarykin), F. N. (1863), The roots of the lymph vessels in the kidneys of mammals. "Med. vestn." 26; "Voyen. med. zh." 6, 168.

Zhdanov, D. A (1934), Contributions to some histophysiological peculiarities of the lymph-vessel walls. "Trudy I Vseross. gistol. konf." 207.

Zhdanov, D. A (1940a), Functional anatomy of the lymph system. "Izd. Gorkovsk. med. in-ta." Gorky

Zhdanov, D A (1940b), Pathways of reabsorption from the pleural cavities. "Probl. tuberk" 11, 56

Zhdanov, D. A (1942a), About the role of blood and lymph vessels of the omentum in the reabsorption of true and colloid solitions and suspensions from the abdominal cavity. "Sb. Materialy k anatomii limfaticheskih sosudov i uzlov." Gorky.

Zhdanov, D. A. (1942b), The right lymphatic duct and its roots. "Sb. Materialy k anatomii limfaticheskih sosudov i uzlov." Gorky.

Zhdanov, D. A. (1945), Anatomy of the thoracic duct and of the main lymph collectors of the trunk. "Trudy Tomskogo med. in-ta" 14, 3.

Zhdanov, D. A (1946), Reabsorption pathways of solutions and suspensions from the serous cavities. "Sb Voprosy hirurgii voyn. i abdominalnoy hirurgii."

Zhdanov, D A. (1948), Data to the anatomy of the lymph vessels of the esophagus. Sb trudov, posv. prof. A. G. Savinyh." Tomsk.

Zhdanov, D A. (1949a), The connections of the lymphatic system of stomach with the lymphatics of the adjacent organs. Vrach. delo" 1015.

Zhdanov, D. A (1949b), Data to the history of the researches made on the main lymph trunks "Trudy Leningr. san.-gig. med. in-ta" 3, 14.

Zhdanov, D A. (1951), New data to the anatomy of the lymphatic system of human skin. "Trudy Leningr. san-gig. med. in-ta" 9, 5.

Zhdanov, D A (1952), General anatomy and physiology of the lymphatic system "Medgiz", Leningrad.

Zhdanov, D. A (1953a), Anastomoses and confluences of the lymphatic system of stomach and duodenum of man. "Trudy Leningr. san.-gig. med. in-ta" 17, 103.

Pozharski, P., N. Toltchskaya & A. Shilova (1950), Pathological anatomy of the silicosis. "Arh. patologii" 2, 13.

Prives, M. G (1948), Roentgenography of the lymphatic system. Leningrad.

Prives, M. G. (1955), Collateral lymph circulation in case of lesions of the nervous system. "Arh. anat. gistol. i embriol." 3, 74, 82

Rabinovich, E. A. (1935), Peculiarities of the lymph circulation of visceral pleura and lung in advanced age. "Probl. tuberk." 9, 40.

Ravitch-Shtcherbo, V. A. (1940), On the physiological retrograde lymph-flow of the endothoracic area. "Sb. Retrogradny limfotok endotorakalnoy oblasti." Voronezh.

Reingold, M. L (1941), To the problem of the retrograde mediastino-cardinal flow of the lymph. "Sb. Retrogradny limfotok endotorakalnoy oblasti" Voronezh.

Rindovski, D F. (1871), The lymph vessels of the kidneys. "Trudy III cyezda russkih yestestvoispytateley. Otd. nauchn. med." 57.

Rotenberg, A. L. (1949), Confluence of efferent lymph vessels of liver and stomach. "Trudy Leningr. san.-gig med. in-ta" 3, 160.

Rotenberg, A L (1952), Intra-organic lymphatic system of the lung "Trudy Leningr. san.-gig. med. in-ta" 17, 14.

Rusznyák, I. (1954), New researches in the physiology and pathology of the lymph circulation. "Klinich. Medits." 32/1, 23.

Shaklein, I. A. (1950), Significance of the pulmonary lymphatic system at healing pneumothorax "Probl. tuberk." 1, 31.

Stefanis, F. A. (1902), On the lymph vessels of the human kidneys Kiev.

Shtefko, V. G. (1935), Pathological anatomy of the lymphatic phases of tuberculous processes of the lung. "Probl. tuberk" 9, 20.

Shtefko, V G. (1937), Problems of pathological anatomy and clinics of the lymphatic phases of tuberculous processes of the lung "Probl. tuberk." 7.

Shtefko, V. G. (1938), Pathological anatomy of pulmonary tuberculosis and of tuberculosis of bones and joints. Moscow

Sikorski, N. (1872), On the lymph vessels of the lung. Kiev.

Skvortsov, Y. Y. (1874), Materials to the anatomy and histology of the pericardium of man. Saint Petersburg

Spirov, M. S (1937), Efferent lymph vessels of the human thyroid gland and their anatomical relationships with the efferent lymph vessels of the trachea. "Sb. trudov, posv. 40-letiyu nauchn. deyateln. U. N Shevkunenko."

Spirov, M. S (1953), Criticism of Prof D A. Zhdanov's book· General anatomy and physiology of the lymphatic system. Moscow 1952. "Medgiz, Arhiv. anatomii, gistologii i embriologii" 30/3, 78.

Stein (Shteyn) T. A. (1948), Contributions to the pathological anatomy of the lobular pneumonia of children. "Arh patologii" 10, 75.

Strukov, A Y (1933), Fundamental principles of the histostructure of the lung. "Osnovy vozrastnoj morfologii" 9.

Strukov, A. Y. (1948), Forms of pulmonary tuberculosis in the light of the morphology

Sushko, A A. (1937), Morphology of lymph flow in man. "Eksperiment Meditsina Kiev" 1/19

Sushko, A. A. (1950), Are there direct communications between the lymphatic and venous vessels in the organs? "Sb posv. 50-letiyu nauchn. deyateln A. P. Krymova, Kiev."

Sysganov (Sisganow), A. N. (1940), On the lymph vessels of the human kidneys and the capsule. Alma-Ata.

Ter-Grigorova, E. N. (1951), Morphological alterations of the liver in Botkin's disease (epidemic hepatitis) at its dynamic development. "Pat. anat inf. bol." Moscow 50.

Timofeyev, D. A. (1896), On the nerve endings in the male genital organs of man and mammalia. Kazan.

Troitskaya, A. A (1946), The lymph vessels of diaphragm. "Vestn hir. i pogran. obl." 6/16, 35.

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of the vegetat[illegible]
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